

-FINAL-

July 2012 [Revision 2]

**SAMPLING AND ANALYSIS PLAN/
QUALITY ASSURANCE PROJECT PLAN
OPERABLE UNIT 3, LIBBY ASBESTOS SUPERFUND SITE
Phase V, Part B: 2012 Ecological Investigations**

Prepared for and with oversight by:



**U.S. ENVIRONMENTAL PROTECTION AGENCY
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SAP/QAPP for OU3, Libby Asbestos Superfund Site
Phase V, Part B: 2012 Ecological Investigations

Revision Log:

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0	04/20/12	--
1	05/22/12	<ul style="list-style-type: none"> • Modify Section 2 to address laboratory conflict of interest issue; add asbestos analytical laboratory selection criteria (Appendix G); remove any text references to EMSL • Add Troy SPF audit text to Section 11.1 • Added a settling time for water samples with heavy sediment loading (see Modification LFM-OU3-01) • Change reference location for caged fish studies from Bobtail Creek tributary to Noisy Creek (see Modification LFM-OU3-02); update protocol to reflect this change (i.e., create protocol revision 1) • Figure 2-1: Add SRC and correct SPF box color
2	7/20/12	<ul style="list-style-type: none"> • Added Section 7 (Fish Tissue Burden Assessment) and make necessary edits to other sections [Sections 1.0, 1.4, 8.6.6, 8.6.7, 8.7, 9.1.2, and 12.1.2] associated with the addition of this study • Added Appendix A.5 (Fish Tissue Collection Protocol) • Added footnote to clarify use of Libby Laboratory Modification #LB-000066d (per Modification LFM-OU3-03) • Corrected subsection header numbering in Section 8

SAP/QAPP for OU3, Libby Asbestos Superfund Site
Phase V, Part B: 2012 Ecological Investigations
Revision 2 - July 2012

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***The most recent versions of field SOPs, FSDS forms, and COC forms are provided electronically in the OU3 eRoom (<https://team.cdm.com/eRoom/mt/LibbyOU3>). The most recent versions of laboratory SOPs are provided electronically in the Libby Lab eRoom (<https://team.cdm.com/eRoom/mt/LibbyLab>).*

List of Abbreviations and Acronyms

α	alpha
%	percent
<	less than
μm	micrometer
Ago	area of a grid opening
ANOVA	analysis of variance
AOC	Administrative Order on Consent
BERA	baseline ecological risk assessment
BTAG	Biological Technical Assistance Group
BTT	Bobtail Creek tributary
CCV	continuous calibration verification
CHISQ	chi-squared
CI	confidence interval
cm	centimeter
COC	chain of custody
DO	dissolved oxygen
DQO	data quality objective
EDD	electronic data deliverable
EDS	energy dispersive spectrometry
EFA	effective filter area
EPA	U.S. Environmental Protection Agency
FEL	Fort Environmental Laboratories, Inc.
FS	feasibility study
FSDS	field sample data sheet
FTL	field team leader
ft/s	feet per second
GC/MS	chromatography/mass spectrometry
Golder	Golder Associates Inc.
GOx	number of grid openings
GPS	global positioning system
HASP	health and safety plan
HAZWOPER	Hazardous Waste Operations and Emergency Response
HDPE	high-density polyethylene
H&S	health and safety
ICV	initial calibration verification
ID	identification
IDW	investigation-derived waste
IS	internal standard
ISO	International Organization for Standardization

KDC	Kootenai Development Corporation
L	liters
L ⁻¹	1/liters
LA	Libby amphibole
LC	laboratory coordinator
LCS	laboratory control sample
LCSD	laboratory control sample duplicate
LRC	lower Rainy Creek
MDEQ	Montana Department of Environmental Quality
MFWP	Montana Department of Fish, Wildlife and Parks
mL	milliliter
mm	millimeter
mm ²	square millimeters
MMT	median metamorphosis time
MS	matrix spike
MSD	matrix spike duplicate
N	number of structures
NFG	National Functional Guidelines
NIOSH	National Institute of Occupational Safety and Health
NIST	National Institute of Standards and Technology
NSY	Noisy Creek
NVLAP	National Voluntary Laboratory Accreditation Program
OSHA	Occupational Safety and Health Administration
OU	operable unit
OU3	Operable Unit 3
PDF	portable document format
PE	performance evaluation
PLM	polarized light microscopy
PLM-VE	polarized light microscopy, visual area estimation
PR	percent recovery
PRI-ER	Project Resources, Inc. - Environmental Restoration
QA	quality assurance
QAM	quality assurance manager
QAPP	quality assurance project plan
QATS	Quality Assurance Technical Support
QC	quality control
Remedium	Remedium Group, Inc.
RF	response factor
RI	remedial investigation
ROM	record of modification
RPD	relative percent difference
RPM	Remedial Project Manager

S	biologically significant amount
SAP	sampling and analysis plan
Shaw	Shaw Environmental, Inc.
Site	Libby Asbestos Superfund Site
SOP	standard operating procedure
SPF	Sample Preparation Facility
SVL	snout-vent length
TAS	target analytical sensitivity
TEM	transmission electron microscopy
TTM	time to metamorphosis
URC	upper Rainy Creek
USGS	U.S. Geological Survey
V	volume
WRS	Wilcoxon Rank Sum
YOY	young of year

1.0 Introduction

This document contains the elements required for both a sampling and analysis plan (SAP) and quality assurance project plan (QAPP). This SAP/QAPP describes studies that will be implemented in 2012 as part of the remedial investigation (RI) currently being performed at Operable Unit 3 (OU3) of the Libby Asbestos Superfund Site (Site). The primary objective of these studies is to gather data to support a baseline ecological risk assessment (BERA) for OU3.

1.1 Site Description

Libby is a community in northwestern Montana that is located near a large open-pit vermiculite mine. Vermiculite from the mine at Libby is known to be contaminated with amphibole asbestos that includes several different mineralogical classifications, including richterite and winchite, with lower frequencies of tremolite, edenite, magnesioriebeckite, and magnesioarfvedsonite (Meeker *et al.* 2003). Depending on the valence state of iron, some particles may also be classified as actinolite. For the purposes of the U.S. Environmental Protection Agency (EPA) investigations at the Site, this mixture is referred to as Libby amphibole (LA).

Historic mining, milling, and processing of vermiculite at the Site are known to have caused releases of vermiculite and LA to the environment. Given the size and complexity of the Site, the EPA has designated a number of operable units (OUs) to facilitate investigations at the Site. **Figure 1-1** shows a preliminary study area boundary for OU3. OU3 includes the property in and around the former vermiculite mine and the geographic area surrounding the mine that has been impacted by releases and subsequent migration of hazardous substances and/or pollutants or contaminants from the mine. The EPA established the preliminary study area boundary for the purpose of planning and developing the scope of the RI for OU3. This study area boundary may be revised as data are obtained on the nature and extent of environmental contamination associated with releases that may have occurred from the mine site. The final boundary of OU3 will be defined by the final EPA-approved RI.

1.2 Basis for Concern at OU3

1.2.1 Human Health Risk

Inhalation of LA associated with this vermiculite is known to have caused a range of adverse health effects in exposed humans, including workers at the mine and processing facilities (Amandus and Wheeler 1987, McDonald *et al.* 1986, McDonald *et al.* 2004, Sullivan 2007, Rohs *et al.* 2007), as well as residents of Libby (Peipins *et al.* 2003). Based on these adverse effects, the EPA listed the Site on the National Priorities List in October 2002.

1.2.2 Ecological Risk

OU3 contains habitat that support a wide variety of wildlife. This includes terrestrial receptors (birds, mammals) that may be exposed on the mine site and in the forested area surrounding the mine, and aquatic receptors (fish, aquatic invertebrates, amphibians) that may be exposed in ponds and streams that are present in OU3. The potential adverse effects of asbestos on wildlife and aquatic receptors are not as well studied; however, due to the extent of contamination, the EPA is concerned that LA might pose a risk to some ecological receptors.

1.3 Summary of Ecological Investigations Performed to Date

To date, the EPA has planned and performed¹ a number of activities to characterize the nature and extent of environmental contamination in OU3 and to collect data to allow the EPA to evaluate risks to ecological receptors. In general, the EPA is seeking to obtain data from multiple lines of evidence to support risk-management decisions at the site. Data collected to date are summarized below.

1.3.1 Nature and Extent

The EPA has collected and analyzed a wide variety of environmental samples to help characterize the nature and extent of environmental contamination in OU3. This includes samples of the following media:

- Ores, soils, and mine wastes that are present at the mine
- Soils in the forested area surrounding the mine
- Duff and tree bark from the forested area surrounding the mine
- Surface water and sediment from streams, rivers, and ponds in OU3

These studies have revealed that, as expected, LA is present in relatively high concentrations in some ores and soil samples from the mine site, and that LA is also present in duff, soil, and tree bark in the surrounding forest, with a general tendency for concentrations to be highest near the mine and downwind of the mine (to the north-northeast), with concentrations generally tending to decrease as a function of distance. These studies also reveal that LA is present in sediment and surface water of streams, rivers, and ponds in OU3.

¹ Field investigations that are planned by the EPA are implemented by respondents W.R. Grace & Co. - Conn. and Kootenai Development Corporation under the oversight of the EPA.

1.3.2 *Small Mammal Lesion Study*

In order to investigate if LA was causing observable effects on small mammals living downwind of the mine, mice were collected and examined for external and internal lesions that might be associated with exposure to LA. The study did not detect any increase in the frequency of lesions compared to mice from a reference area, indicating that exposures of short-lived small mammalian receptors in the forested area around the mine are not likely to be of concern.

Although avian-specific studies have not been performed, some experts suggest that birds are not likely to be more susceptible to LA exposures relative to small mammals (Wideman 2011). Consequently, the EPA determined that field studies of bird toxicity are not needed.

1.3.3 *Aquatic Toxicity Testing*

The EPA has performed two studies to investigate the effects of site water and sediment on aquatic receptors.

In the first study, rainbow trout fry were exposed to LA in water derived from the site for 40 days (Parametrix 2009b). Although no adverse effects were observed, it was subsequently determined that LA in the water became clumped by organic material in the water, and these clumps adhered to the walls of the exposure aquaria, reducing exposure concentrations to low levels. Consequently, this study does not provide meaningful results regarding the potential toxicity of LA on fish.

In the second study, the EPA evaluated risks from sediments to two aquatic invertebrate species (*Hyalella azteca* and *Chironomus tentans*). Sediments were collected from two onsite sampling locations and from two reference sites. Measurement endpoints included survival, growth, and reproduction. Neither test organism exhibited any statistically significant difference in survival, growth, or reproduction when compared to both laboratory control sediments and field-collected reference sediments (Parametrix 2009c, 2009d). These results suggest that exposure to LA or other contaminants at the levels present in the onsite test sediments does not adversely impact aquatic invertebrates.

1.3.4 *Aquatic Population Surveys*

The EPA has performed two demographic studies of fish and aquatic invertebrates in lower Rainy Creek (LRC) and compared the observations from LRC to results from appropriate reference streams. These studies indicated that aquatic invertebrates in LRC are not substantially impacted compared to reference streams (Parametrix 2010), but that the density of trout in LRC is lower than expected by comparison to upper Rainy Creek (URC) and other nearby reference streams (i.e., Noisy Creek [NSY], Bobtail Creek tributary [BTT]) (Parametrix

2009a, 2010). Efforts to determine whether these apparent population differences in trout are attributable to habitat differences or to an adverse effect of LA have not been definitive (Parametrix 2009a).

1.4 Scope of Ecological Studies Planned for 2012

Based on the studies performed to date, the chief question that the EPA is seeking to resolve is whether LA may be contributing to the apparent decrease in fish density in LRC. This document includes two studies designed to help address this question. In addition, because no studies have yet been performed to evaluate risks to amphibians from LA in onsite water and sediment, two studies are planned to investigate whether LA may be of concern to this class of receptor. Finally, because fish may tend to retain LA in their tissues, one study is planned to measure the concentration of LA in the tissues of fish captured on site.

1.5 Organization of this Document

As noted above, this document is a SAP/QAPP that describes the data collection efforts for the ecological studies described above. This SAP/QAPP has been developed in basic accordance with the *EPA Requirements for Quality Assurance Project Plans*, EPA QA/R-5 (EPA 2001) and the *Guidance on Systematic Planning Using the Data Quality Objectives Process – EPA QA/G4* (EPA 2006). While this SAP/QAPP is organized differently from the recommended structure in the QA/R-5 guidance, all the required QAPP elements are presented. **Table 1-1** provides a cross-reference where information for each QA/R-5 element is located in this SAP/QAPP. This document is organized as follows:

- Section 1 – Introduction
- Section 2 – Study Roles and Responsibilities
- Section 3 – Amphibian Laboratory Toxicity Testing
- Section 4 – Amphibian Field Study
- Section 5 – In-Stream Caged Fish Studies
- Section 6 – Native Fish Lesion Study
- Section 7 – Fish Tissue Burden Assessment
- Section 8 – General Field Procedures and Requirements
- Section 9 – Sample Preparation and Analytical Requirements
- Section 10 – Data Management
- Section 11 – Assessment and Oversight
- Section 12 – Data Validation and Usability
- Section 13 – References

All cited tables, figures, and appendices are located at the end of this document.

2.0 Study Roles and Responsibilities

Figure 2-1 presents an organizational chart that illustrates the lines of authority and communication between the agencies and contractors for this project. The following sections summarize the entities and individuals that will be responsible for providing project management, SAP/QAPP development, field sampling support, onsite field coordination, laboratory support, data management, and quality assurance (QA) for this project.

2.1 Project Management

The EPA is the lead regulatory agency for Superfund activities within OU3. The EPA Remedial Project Manager (RPM) for OU3 is Christina Prograss, EPA Region 8. Ms. Prograss is a principal data user and decision-maker for Superfund activities within OU3. The EPA ecological risk assessor for the project is Dan Wall, EPA Region 8. The project is also supported by the OU3 Biological Technical Assistance Group (BTAG), which is composed of several scientists from a variety of disciplines (e.g., biology, ecology, risk assessment) and agencies that advise the RPM on the ecological risk assessment matters.

The Montana Department of Environmental Quality (MDEQ) is the support regulatory agency for Superfund activities within OU3. The MDEQ Project Manager for OU3 is John Podolinsky. The EPA will consult with MDEQ as provided for by the Comprehensive Environmental Response, Compensation, and Liability Act, the National Contingency Plan, and other applicable guidance in conducting Superfund activities within OU3.

The EPA has entered into an Administrative Order on Consent (AOC) with Respondents W.R. Grace & Co.-Conn. and Kootenai Development Corporation (KDC) for performance of a remedial investigation/feasibility study (RI/FS) at OU3 of the Libby Asbestos Site. Under the terms of the AOC, W.R. Grace & Co.-Conn. and KDC will implement this SAP/QAPP. The designated Project Coordinator for Respondents W.R. Grace & Co.-Conn. and KDC is Robert Medler of Remedium Group, Inc. (Remedium). Remedium has chosen the following subcontractors to implement this SAP/QAPP:

- Golder Associates Inc. (Golder)
- Chapman Construction, Inc.
- Anchor QEA
- Energy Laboratory
- Fort Environmental Laboratory (FEL)
- Northwest ZooPath

2.2 SAP/QAPP Development

This SAP/QAPP was developed by CDM Smith and SRC, Inc. at the direction of and with oversight by the EPA. Copies of the SAP/QAPP will be distributed by the CDM Smith Project Manager (or their designate), either in hard copy or in electronic format, as indicated in the distribution list. The CDM Smith Project Manager (or their designate) will distribute updated copies each time a SAP/QAPP revision occurs. A copy of the final, signed SAP/QAPP (and any subsequent revisions) will also be posted to the OU3 website² and the OU3 eRoom³.

2.3 Field Sampling Support

All field collection activities described in this SAP/QAPP will be performed by Remedium and their contractors, in accordance with this SAP/QAPP. On behalf of Remedium, all field work will be performed by Golder Associates Inc. (Golder) or their subcontractors. Golder will conduct the field work with additional field logistical support provided, as needed, by Chapman Construction, Inc. and Anchor QEA. The individuals responsible for field implementation and oversight of each study are identified below:

Amphibian Studies and In-Stream Caged Fish Studies

- Project Manager: Sue Robinson
- Field Team Leader: Jeremy Clark (designee Traci Sanderson)
- Field Data Coordinator: Traci Sanderson
- Field Quality Assurance Manager: Jeremy Clark
- Field Health and Safety Managers: Jeremy Clark, Traci Sanderson

Resident Trout Lesion Study and Fish Tissue Study

- Project Manager: Sue Robinson
- Field Team Leader: Joe Volosin
- Field Data Coordinator: Joe Volosin
- Field Quality Assurance Manager: Jeremy Clark
- Field Health and Safety Managers: Joe Volosin, Jared Simpson

2.4 Onsite Field Coordination

Access to the mine and other areas of OU3 via Rainy Creek Road is currently restricted and is controlled by the EPA. The onsite point of contact for access to the mine is Rob Burton of Project Resources, Inc. - Environmental Restoration (PRI-ER):

rob.burton@priworld.com
(406) 293-3690

² <http://cbec.srcinc.com/libby/>

³ <https://team.cdm.com/eRoom/mt/LibbyOU3>

2.5 Laboratory Support

2.5.1 *Toxicity Testing and Histological Support*

Amphibian toxicity testing will be performed at a laboratory chosen by Remedium (i.e., Fort Environmental Laboratories, Inc. [FEL] in Stillwater, Oklahoma). Gross and histological examination of fish and amphibians collected from the site and reference areas will be conducted by a laboratory chosen by Remedium (i.e., Northwest ZooPath laboratory in Monroe, Washington.) Golder, on behalf of Remedium, is responsible for laboratory procurement, establishing subcontracting agreements with Remedium-chosen laboratories, and managing test conduct.

2.5.2 *Analytical Support*

Asbestos

All samples collected as part of this project for asbestos analysis will be sent for preparation and/or analysis at laboratories that meet the Libby-specific laboratory criteria that have been established for the project. These criteria are specified in **Appendix G**. Remedium may choose whether asbestos analytical laboratory services are procured directly or if services will be provided via EPA.

Unless Remedium identifies a suitable sediment sample preparation laboratory that meets the requirements set forth in **Appendix G**, sediment samples for asbestos analysis will be prepared (dried, sieved, ground) at the Sample Preparation Facility (SPF) in Troy, Montana. The SPF is managed by the EPA Environmental Services Assistance Team contractor, TechLaw, Inc.

Non-Asbestos

All samples collected as part of this project for non-asbestos analysis will be sent to a laboratory chosen by Remedium (i.e., Energy Laboratories, Inc. in Billings, Montana). Remedium is responsible for laboratory procurement and establishing subcontracting agreements with the non-asbestos analytical laboratory.

2.6 Data Management

Administration of the master database for OU3 will be performed by EPA contractors. The primary database administrator will be Lynn Woodbury (CDM Smith). The database administrator (or their designate) will be responsible for sample tracking, uploading new data, performing data verification and error checks to identify incorrect, inconsistent, or missing data, and ensuring that all questionable data are checked and corrected as needed. When the OU3

database has been populated, checked, and validated, relevant asbestos data may be transferred into a Libby Asbestos Superfund Site database, as directed by the EPA for final storage.

2.7 Quality Assurance

There is no individual designated as the EPA Quality Assurance Manager (QAM) for the Libby project. Rather, the Region 8 QA program has delegated authority to the EPA RPMs. This means that the EPA RPMs have the ability to review and approve governing investigation documents developed by Site contractors. Thus, it is the responsibility of the EPA RPM for OU3, who is independent of the entities planning and obtaining the data, to ensure that this SAP/QAPP has been prepared in accordance with the EPA QA guidelines and requirements. The EPA RPM is also responsible for managing and overseeing all aspects of the quality assurance/quality control (QA/QC) program for their respective OUs. In this regard, the RPM is supported by the EPA Quality Assurance Technical Support (QATS) contractor, Shaw Environmental, Inc. (Shaw). The QATS contractor will evaluate and monitor QA/QC sampling and is responsible for performing annual audits of each analytical laboratory.

3.0 Amphibian Laboratory Toxicity Testing

3.1 Data Quality Objectives

Data quality objectives (DQOs) define the type, quality, quantity, purpose, and intended uses of data to be collected (EPA 2006). The design of a study is closely tied to its DQOs, which serve as the basis for important decisions regarding key design features such as the number and location of samples to be collected and the analyses to be performed. The DQO process typically follows a seven-step procedure to ensure that the project plan is carefully thought out and that the data collected will provide sufficient information to support the key decisions which must be made (EPA 2006).

The following sections implement the seven-step DQO process associated with the amphibian laboratory toxicity test.

3.1.1 Step 1: State the Problem

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including several ponds within OU3. Amphibians may be exposed to LA in the aquatic environment (including exposure to both surface water and sediment), and also in the terrestrial environment (soil). Of these two environments, it is suspected that the highest exposure and the greatest susceptibility is likely to occur during the early (aquatic) life stages of this receptor group, so attention is focused on aquatic media (i.e., surface water and sediment). Amphibians may be exposed to LA in these environmental media during their aquatic life stage via direct contact and ingestion.

Ecological risk assessments typically utilize an approach that evaluates multiple lines of evidence to draw conclusions about potential adverse effects to the population of interest. At present, there are no data from OU3 to support any of the lines of evidence potentially useful for evaluating the risks to amphibians from LA in surface water or sediment. As part of the RI at OU3, the EPA is pursuing two lines of evidence to help evaluate if exposure of amphibians to LA is of significant ecological concern, including the following:

1. A laboratory-based study of effects in developing amphibians exposed to sediment collected from a contaminated onsite location
2. A field study of the occurrence of any abnormalities in amphibian species in onsite pond areas (see Section 4)

This section provides the DQOs for the first line of evidence.

3.1.2 Step 2: Identify the Goal of the Study

In site-specific laboratory toxicity studies, test organisms are exposed to site media in a laboratory setting and measurements are made of particular endpoints of interest to determine if exposures are having an adverse impact.

Ideally, because amphibians may be exposed via surface water and sediment, it would be desirable to include both exposure media in the test conditions. However, previous attempts at surface water toxicity tests have shown that it is very difficult to maintain exposure conditions for LA in surface water, because the fibers tend to become bound to organic matter in the water column and on the walls of the exposure chambers. Thus, this study will focus only on an evaluation of potential exposures to LA in sediment.

The goal of the amphibian laboratory toxicity test is to determine if exposure of amphibians to LA in sediment from OU3 will result in adverse effects on survival, growth, or metamorphosis. Note that reproduction was considered as a potential endpoint, but the length of time required to assess this endpoint (i.e., 5-6 additional months of exposure), and resources needed to complete a full reproduction study were determined to be impractical to implement. However, potential effects on presumptive gonad tissue may be examined as an indirect way to evaluate the reproductive endpoint if the results of the amphibian field study (see Section 4) show potential effects on development (i.e., primary differentiation) of gonad tissues.

3.1.3 Step 3: Identify the Types of Data Needed

The information inputs that are needed to achieve the study goal include reliable measures of endpoints related to survival, growth, and metamorphosis in developing amphibians exposed to LA in sediment that are at the high-end of the range of LA concentrations observed in OU3. Analogous data from amphibians exposed to uncontaminated sediments are also needed to allow for comparisons between contaminated and uncontaminated locations.

3.1.4 Step 4: Define Study Boundaries

Spatial Bounds

The laboratory toxicity tests should be conducted with site sediment collected from OU3 at a location containing concentrations of LA that are consistently reported to be at the high-end of the range detected at the Site. The maximum LA concentrations measured in OU3 streams and ponds by polarized light microscopy using visual area estimation (PLM-VE) is about 2-4 percent (%).

Sediments collected from station TP-TOE2 (see **Figure 3-1**), located in LRC between the Tailings Impoundment and the Mill Pond, have consistently yielded LA concentrations of 2-3% across

multiple years of sampling. Although station TP-TOE2 may not provide suitable habitat for amphibians, this station provides levels of LA in sediment that are consistently high over time and similar to the highest concentrations measured in sediments of the ponded areas of OU3 (which can range from trace levels to 2% depending upon the location in the pond). Sediment samples from Carney Creek (CC-1) also tend to have high levels of LA over time, with 3-4% being reported in the studies conducted in 2007 and 2008. Based on this, site sediments from TP-TOE2 or CC-1 would be appropriate for use in the laboratory toxicity tests.

In addition, sediments should also be collected from a pond in a reference area for use in the laboratory toxicity tests as an uncontaminated reference sediment.

Temporal Bounds

The concentration of LA in sediments is not expected to vary greatly by time. Thus, the timing of sediment collection is primarily based on ease of sample collection. However, the timing of the exposure study does depend on the availability of egg masses of a suitable test species, and this occurs in the spring. Therefore, site sediments should be collected in April 2012 to allow time for testing prior to selection and shipment of the test sediment to the toxicity laboratory.

3.1.5 Step 5: Measurement Endpoints and Data Interpretation

This study will measure ecologically relevant endpoints in amphibians exposed to LA in sediment at concentrations that represent the high-end of site contamination conditions and determine if these endpoints are statistically different from those measured in organisms exposed to uncontaminated sediment. The following table summarizes the measurement endpoints and their relation to the assessment endpoints:

Assessment Endpoint	Measurement Endpoint
Survival	<ul style="list-style-type: none"> - % mortality - Presence of abnormal behavior that could affect survival
Growth	<ul style="list-style-type: none"> - Whole body weight - Snout-vent length (SVL)
Metamorphosis	<ul style="list-style-type: none"> - % completing metamorphosis - Time to metamorphosis (TTM) - Median metamorphosis time (MMT) - Hind limb length (normalized to SVL), if metamorphosis does not occur
Abnormalities/ Malformations*	<ul style="list-style-type: none"> - Incidence of malformations that could affect survival or growth - External abnormalities (i.e., eyes, mouth, torso, hind limbs) - Internal abnormalities (i.e., liver, kidneys, heart, lung, presumptive gonad)
Histopathology (if determined to be necessary)	<ul style="list-style-type: none"> - Presumptive gonad tissue - Thyroid gland - Blood measures of thyroid hormones

*Assessment of abnormalities and malformations should be performed on all organisms regardless of Gosner stage at study termination.

The precise statistical tests that will be used to compare exposed and control organisms will vary between the measurement endpoints. For discrete endpoints (survival, malformation frequency), it is expected that comparisons will be made using the Fisher Exact test. For continuous endpoints (e.g., body weight, MMT), it is expected that the comparisons between control and treated groups will be performed using the Wilcoxon Rank Sum (WRS) Test (unless the data are distributed approximately normally, in which case comparisons may be performed using t-statistics). Other statistical tests that may be appropriate include one-way analysis of variance (ANOVA) or an ANOVA on ranks. *Post hoc* tests may also be used such as Dunnett's test or Bonferroni t-test for parametric sets, or Dunn's test for non-parametric tests.

If no statistically significant differences in any of the endpoints are detected between the exposed and the control organisms, it will be concluded that exposure to contaminants in sediment at concentrations equal to or less than the levels tested are not likely to cause effects that are ecologically significant. If statistically significant changes in one or more measurement endpoints are observed, additional investigation may be needed to determine if those effects result in ecologically significant effects at the population level, and to identify a no-effect level and potentially a low-effect level (depending upon the degree of effect severity observed in the toxicity test) that may be used to evaluate remedial alternatives. However, the potential need for additional studies will depend in part on the results from other lines of evidence being collected to evaluate risks to amphibians (see Section 4).

3.1.6 Step 6: Specify Performance or Acceptance Criteria

In evaluating the results of amphibian toxicity testing, two types of decision errors are possible:

- A *false negative decision error* occurs when it is decided that there are no significant effects on amphibians, when in fact there are.
- A *false positive decision error* occurs when it is decided that there are significant effects on amphibians, when in fact there are not.

The probability of decision errors when comparing two data sets (site *vs.* reference) is controlled by the selection of the null hypothesis, and by selection of an appropriate statistical method to test the null hypothesis (EPA 2002). Two alternative forms of null hypothesis are possible:

- Form 1: The null hypothesis is that no difference exists between site and reference. A confidence level of 100(1- α)% is required before the null hypothesis is rejected and it can be declared that the site data are higher than the reference data.
- Form 2: The null hypothesis is that the site is higher than reference by some amount (S) that is considered to be biologically significant. A confidence level of 100(1- α)% is

required before the null hypothesis is rejected and it is declared that the difference between site and reference, if any, is smaller than S.

For the purpose of this effort, the Form 1 null hypothesis is selected for use, because it is the most familiar, is the easiest to interpret, and does not require specification of an effect that is presumed to be significant. In accordance with EPA (2002), when the Form 1 null hypothesis is used, it is appropriate to select a value of α that is somewhat higher than the usual value of 0.05, such that marginal differences between site and reference are more easily identified as being significant. Therefore, α is set to 0.20.

3.1.7 Step 7: Optimize the Study Design

Based on the DQOs presented above, the following are key study design features of the amphibian toxicity test.

Study Design

The study design will consist of three exposure groups, as follows:

- Group 1 – Synthetic sediment (laboratory control) plus laboratory water
- Group 2 – Reference (uncontaminated) field sediment plus laboratory water
- Group 3 – Contaminated field sediment (approximately 2-3% LA) plus laboratory water

Each exposure group will consist of four replicate exposure chambers each containing 20 organisms (i.e., 80 organisms per exposure group). Embryos will be assigned to exposure chambers at random. The study protocol will specify how embryos will be assigned to control/treatment groups.

Exposure chambers will be 2.5-gallon aquaria fitted with standpipes to provide a tank volume of 6 liters. Water in the exposure chambers will be renewed using a flow-through system, but sediment will not be changed. The water renewal rate will be specified in the study protocol. To minimize the potential for LA fiber loss due to water renewal, the renewal rate should be set to the minimum that can maintain acceptable water quality.

The test sediments will be added to each tank and will cover the bottom to a depth of 2 centimeters (cm), with an overlying water depth of 25-30 cm. The expected volume of sediment required for each exposure tank is approximately one liter. The study protocol will specify how water and sediment will be added to the aquaria and how system will be allowed to equilibrate before organisms are introduced. Feeding of organisms will be *ad libitum* and cleaning of tanks will occur daily. The details of how the tanks will be cleaned (particularly any measures to mitigate fiber loss) will be addressed in the study protocol.

Test Materials

Three separate “lots” of contaminated field sediment will be collected from station TP-TOE2 and CC-1 (see **Figure 3-1**), since previous RI sampling results indicate sediment concentrations of about 2-3% at these locations. Each lot of sediment will be a sufficient quantity for the complete amphibian toxicity testing investigation (approximately 15 liters) plus 2 additional liters for pre-testing analysis of LA and non-asbestos analytes, for a total of 17 liters per lot. Five replicate samples from each lot will be analyzed for LA by polarized light microscopy (PLM) (see Section 9.2) to estimate the concentration of LA in each sediment lot. Each sediment lot will also be analyzed for non-asbestos analytes (see Section 9.3). The sediment lot having the consistently highest LA concentration will be used for the amphibian toxicity testing.

The source for the uncontaminated (reference) field sediment will be a pond located near the toxicity testing laboratory in Oklahoma. The synthetic sediment will be clean sand that the laboratory uses for control purposes.

Test Species and Life Stage

Based on onsite observations and data available for Lincoln County, Montana, there are four frog and toad species identified as potentially occurring at OU3 including the western toad (*Bufo boreas*), the Columbia spotted frog (*Rana luteiventris*), the Rocky Mountain tailed frog (*Ascaphus montanus*), and the Pacific treefrog (*Pseudacris regilla*). However, none of these species are available from commercial sources for use in toxicity testing, and the collection of egg masses onsite for laboratory testing is not considered feasible. Several *Ranid* species are available commercially for use in toxicity testing, including the Southern leopard frog (*Rana sphenoccephala*), the Northern leopard frog (*Rana pipiens*), the green frog (*Rana clamitans*), and the wood frog (*Rana sylvatica*). The test species will be one of these *Ranid* species, because they are good surrogates for the Columbia spotted frog (*Rana luteiventris*) present on the site and are also surrogates for the other North American species present onsite. Northern leopard frog (*Rana pipiens*) will be the preferred test species. If *Rana pipiens* eggs are not available then the following will be used in order of preference: Southern leopard frog (*Rana sphenoccephala*) and green frog (*Rana clamitans*). Bullfrogs (*Rana catesbeiana*) will not be used because they are considered to be more tolerant in comparison to the other ranid species. The source of the test species will be identified in the study protocol.

Egg masses will be cultured and free-swimming larva (Gosner stage 20, see **Figure 3-2**) will be tracked until 100% of the control animals complete metamorphosis (Gosner stage 46, see **Figure 3-2**). This is expected to require approximately 45 to 60 days.

Measurements Performed During the Study

Water Quality Measurements

Temperature in the overlying water will be measured daily. Dissolved oxygen (DO), pH, and light intensity will be measured three times per week. Total hardness, alkalinity, conductivity, total residual oxidants, and ammonia-nitrogen will be measured at the beginning and the end of the study. See Section 9.3 for detailed information on water quality analyses.

Overlying water will not be analyzed for LA.

Biological Measurements Obtained During the Study

All animals will be observed daily. Data that will be recorded daily shall include:

- number of surviving organisms
- developmental stage and number of organisms reaching metamorphosis
- other observations on occurrence of malformations or other abnormalities (e.g., behavior)

It should be noted that if survival becomes less than (\leq) 80% in the control groups, the study will be terminated until the cause of mortality in the controls is determined.

Biological Measurements at Study Termination

Study termination is defined as the time at which 100% of the controls have completed metamorphosis (Gosner stage 46). All organisms will be anesthetized at study termination, digitally photographed, weighed, and examined for external abnormalities. Growth will be assessed by measuring organism weight and length (SVL). For each test organism that completed metamorphosis, the TTM will be recorded. The fraction of organisms completing metamorphosis and MMT will be determined for each replicate.

Major internal organs will be inspected for all organisms for developmental stage and appearance, via necropsy of the body cavity (for metamorphosed specimens) or visual observation through the skin (for earlier Gosner stages). Necropsy observations will be recorded and a second set of digital photos taken. Necropsy will also include the collection blood for possible future analysis of thyroid hormone levels. The head will be separated from the body, and both will be preserved for potential future histopathological examination of the thyroid gland and presumptive gonad tissue.

Any organisms that die prior to the study termination will undergo the same procedures.

3.2 Study Protocol

A detailed study protocol for the amphibian sediment toxicity test has been developed by scientists from FEL, which is responsible for implementing this study. This protocol has been reviewed and approved by the EPA, and is attached as **Appendix A.1** to this SAP/QAPP. Protocols in **Appendix A** may change if Remedium selects different asbestos analytical laboratories, in accordance with Section 2.5, *Laboratory Support*, of this SAP/QAPP, and with the requirements specified in **Appendix G**. This protocol will be revised by Remedium (or their contractors) if a different asbestos analytical laboratory is selected in the future.

3.3 Media Sample Collection

As discussed above, sediment will be collected from two onsite stations (TP-TOE2 and CC-1). Detailed sediment sampling methods are provided in the study protocol (see **Appendix A.1**). In brief, the sediment sampling will be performed in basic accordance with the OU3-specific standard operating procedure (SOP) No. 5, *Sediment Sampling* (see **Appendix B**). Three separate lots of surficial sediment will be collected from the onsite stations (1 lot = 17 liters of sediment). Five replicates of each lot will be analyzed for LA by PLM (see Section 9.2). One composite sediment sample from each station will be analyzed for non-asbestos analytes (see Section 9.3). This composite sample will be created by taking equal aliquots from each of the three collected lots for the station. Sediment for non-asbestos analysis will be submitted in four 4-ounce glass jars (see Section 9.3).

At the termination of the toxicity test, sediment samples of the test materials for each replicate of the onsite sediment exposure group will be collected in accordance with the procedures specified in the study protocol (see **Appendix A.1**) and analyzed for LA by PLM (see Section 9.2).

Total hardness, alkalinity, conductivity, total residual oxidants, and ammonia-nitrogen will be measured in the overlying water (see Section 9.3) for each replicate at the beginning and the termination of the toxicity test. The water sampling procedures are detailed in the study protocol (see **Appendix A.1**).

3.4 Field Quality Control Samples

No field-collected quality control (QC) samples are required for this study.

4.0 Amphibian Field Study

4.1 Data Quality Objectives

The following sections implement the seven-step DQO process for studies to assess potential effects of LA in amphibians under-going development in onsite pond habitats.

4.1.1 Step 1: State the Problem

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including sediments and surface waters draining from the mine area. This includes a number of ponded areas that provide suitable habitat for amphibians (frogs, toads). Amphibians may be exposed to LA in the environment by direct contact with LA in both sediment and surface water. As part of the RI at OU3, the EPA is pursuing two lines of evidence to help evaluate if exposure of amphibians to LA in OU3 is of significant ecological concern, including the following:

1. A laboratory-based study of effects in developing amphibians exposed to sediment collected from a contaminated onsite location (see Section 3)
2. A field study of the occurrence of any abnormalities in amphibian species in onsite pond areas

This section provides the DQOs for the second line of evidence.

4.1.2 Step 2: Identify the Goal of the Study

The goal of this study is to determine if the frequency of lesions or abnormalities is higher in amphibians developing in onsite locations containing LA than in amphibians developing in (uncontaminated) reference locations.

4.1.3 Step 3: Identify the Types of Data That Will Be Collected

The data needed for this line of evidence includes measurement of the occurrence (if any), nature, and frequency of abnormalities in amphibians collected from onsite areas compared with that for amphibians of the same species from one or more reference locations.

In addition, data are needed on the concentration of LA in water and sediment at the locations selected for evaluation. These data are not expected to be sufficient for developing an exposure-response relationship or for predicting effects in areas that are not studied, but are important to

document that water and sediment in the areas selected for evaluation are contaminated with LA.

4.1.4 Step 4: Define Study Boundaries

Spatial Bounds

The optimum locations for collecting and observing developing amphibians in the field cannot be specified with certainty until field reconnaissance has been performed. However, onsite target areas that may provide suitable habitat include the Tailings Impoundment, the Mill Pond, and ponded areas on Fleetwood Creek and Carney Creek (see **Figure 3-1**). Off-site reference areas are not yet identified, but will also be selected based on field reconnaissance. The selection of the reference locations for evaluation in the study will depend upon habitat similarity to the site, sediment analysis results⁴, and ease of access. If possible a total of four (minimum of three) reference locations will be identified and used in the study. Potential areas to be considered include the ponds on BTT (see **Figure 4-1**), heritage sites near Libby, and other nearby ponds located outside of the Libby area. If collected data from the reference areas are not sufficient to perform meaningful comparisons to onsite areas, data published in the literature on background abnormality rates may also be considered.

Temporal Bounds

The exact time that amphibians breed and their eggs begin development depends on many environmental factors, especially temperature. Because of this, the timing of the study cannot be specified with certainty, but will be determined by field observations that identify when breeding begins. However, based on available information, it is expected that breeding is likely to occur in May, and the estimated schedule for the field study is May-August. It is anticipated that field sampling efforts may occur every 2 to 3 weeks (or sooner if available) within this time interval to collect specimens from each developmental window (see below), but the actual collection frequency intervals will depend upon meteorological conditions and specimen availability. Sampling time intervals will also be dictated by when tadpoles are nearing Gosner development stages of interest.

4.1.5 Step 5: Measurement Endpoints and Data Interpretation

Because no information has been located on the potential effects of asbestos on amphibians, it is not known what life stage is likely to be most sensitive. Consequently, the field study will seek to evaluate the full developmental period from egg mass through metamorphosis. This will be stratified into four developmental windows, as follows:

⁴ Sediment from candidate reference ponds should be analyzed for LA, metals, and organics (see Section 9) to provide information on potential contamination.

- Egg mass
- Embryo-larval (Gosner stages 21-25, see **Figure 3-2**)
- Hind limb development completion (Gosner stages 37-40, see **Figure 3-2**)
- Metamorphic completion (post-climax) (Gosner stage 46, see **Figure 3-2**)

Measurement endpoints for each developmental window will include external physical examination for abnormalities in the features summarized below:

Developmental Window	External Measurement Endpoints
Egg mass	Structure Cleavage
Larval	Mouth Gills Eyes Skin Tail Limbs
Gosner stage 37-40	Hind limb length (HLL) Snout-vent length (SVL)
Metamorphosed young	Mouth Eyes Skin Limbs Size (weight and SVL)

External developmental examination will be performed in all specimens and all life stages, regardless of species. In addition, necropsy will be performed in all newly metamorphosed specimens. Histopathology will be performed for the one species with the most complete data set at each site⁵, with special attention to gills, mouth, skin, and gonad tissue. Others tissues may be examined, depending on the outcome of external examination and necropsy, focusing on specimens with developmental anomalies.

The frequency of abnormalities in amphibians from each onsite location will be compared to that for organisms from each reference area on a species by species basis. Only species found in both the reference area and the mine site will be compared. It is expected that comparisons of site with reference will be performed using the Fisher Exact test. Other statistical tests that may be appropriate include one-way ANOVA or an ANOVA on ranks. *Post hoc* tests may also be used such as Dunnett's test or Bonferroni t-test for parametric sets, or Dunn's test for non-parametric tests.

⁵ Of the multiple species that will be collected, the one species that has the most complete number of Gosner stages represented will be selected for histopathology examination.

If no statistically significant differences in frequency or severity of lesions or abnormalities are detected between the exposed and the control organisms, this will be interpreted as being consistent with the conclusion that LA and other site-related contaminants in OU3 do not cause adverse effects on amphibian growth and development. If statistically significant increases in the frequency of abnormalities in amphibians from OU3 are observed, additional investigation may be needed to determine the causal factor(s) (LA *vs.* other site-related contaminants), and to determine if those effects are likely to result in ecologically significant effects at the population level. However, the potential need for additional studies will depend in part on the results from the other line of evidence being collected to evaluate risks to amphibians (see Section 3).

4.1.6 Step 6: Specify Performance or Acceptance Criteria

In evaluating the results of these studies of amphibian development, two types of decision errors are possible:

- A *false negative decision error* occurs when it is decided that there are no significant effects on amphibians, when in fact there are.
- A *false positive decision error* occurs when it is decided that there are significant effects on amphibians, when in fact there are not.

The probability of decision errors when comparing two data sets (site *vs.* reference) is controlled by the selection of the null hypothesis, and by selection of an appropriate statistical method to test the null hypothesis (EPA 2002). Two alternative forms of null hypothesis are possible:

- Form 1: The null hypothesis is that no difference exists between site and reference. A confidence level of $100(1-\alpha)\%$ is required before the null hypothesis is rejected and it can be declared that the site data are higher than the reference data.
- Form 2: The null hypothesis is that the site is higher than reference by some amount (S) that is considered to be biologically significant. A confidence level of $100(1-\alpha)\%$ is required before the null hypothesis is rejected and it is declared that the difference between site and reference, if any, is smaller than S.

For the purpose of this effort, the Form 1 null hypothesis is selected for use, because it is the most familiar, is the easiest to interpret, and does not require specification of an effect that is presumed to be significant. In accordance with EPA (2002), when the Form 1 null hypothesis is used, it is appropriate to select a value of α that is somewhat higher than the usual value of 0.05, such that marginal differences between site and reference are more easily identified as being significant. Therefore, α is set to 0.20.

4.1.7 Step 7: Optimize the Study Design

Based on the DQOs presented above, the following are key study design features of the amphibian field study.

Species

The study may include any amphibian species that is found to be present in OU3. Based on available information, it is considered likely that the predominant species will include the spotted frog and the western toad.

Field Reconnaissance

As noted above, exact locations for collecting developing amphibians in the field cannot be specified with certainty until field reconnaissance has been performed. Ambient air temperature will be checked remotely via the Mesowest website⁶ beginning in March. It is expected that breeding will begin when air temperatures approach 50-60 degrees Fahrenheit. This is anticipated to occur in May or June. A site reconnaissance effort will be conducted in March to identify candidate reference locations where breeding amphibians may be present. As noted above, the selection of the reference locations for evaluation in the study will depend upon habitat similarity to the site, sediment analysis results (for both asbestos and non-asbestos analytes), and ease of access. As part of the site reconnaissance effort, sediment samples will also be collected from OU3 ponds for analysis of both asbestos and non-asbestos analytes to provide data for comparability to the candidate reference ponds.

Training

To ensure that field staff are properly trained to observe, identify, and collect amphibian specimens for this study, a three-day training program led by Douglas Fort (FEL) will be held in Libby from March 26 to 29, 2012. Training topics will cover the following:

Day 1: Introduction to amphibian development, identification, field collection techniques, specimen processing techniques, anesthetization, and euthanasia

Day 2: Candidate reference site visits and qualitative habitat evaluation (most expected to be snow-covered)

Day 3: OU3 site visits and reconnaissance, qualitative habitat identification

⁶ <http://mesowest.utah.edu/cgi-bin/droman/mesomap.cgi?state=MT&rawsflag=3>

Target Sample Size per Site

The target number of amphibians per location varies according to developmental window, as follows⁷:

- 4 egg masses per species (if available) for each site
- 40 tadpoles for Gosner stages 21-25 per species (if available) for each site
- 40 tadpoles for Gosner stages 37-40 per species (if available) for each site
- 20 metamorphosed young for Gosner stage 46 per species (if available) for each site

Temperature Monitoring

As noted above, the estimated schedule for the field study is May-August, 2012. During this time, the field teams will visit the onsite and reference locations twice a week to check if specimens from each developmental window are available. Water temperature will be measured and recorded during each of these visits.

Environmental Characterization

At each onsite (OU3) sampling location, water samples should be collected weekly for the analysis of total LA to provide information on potential changes in surface water concentrations throughout the study duration. Collection of water samples will begin at each location once egg masses are confirmed to be present. Because sediments are not expected to vary substantially over time, two samples of sediment should be collected for analysis of LA, the first sample in the early period of development and the second sample near the end of the study. As noted above, it is not expected that these measurements will allow for development of an exposure-response curve, but they are important to document the level of exposure associated with each site.

For reference areas, because it assumed that LA is low or absent in these areas and that temporal variability of LA concentration is not expected, two samples of water and two sample of sediment should be collected for analysis of LA. The first sample of each media should be collected in the early period of development and the second sample near the end of the study.

4.2 Study Design

A detailed study protocol for the amphibian field study has been developed by Remedium-contracted scientists responsible for field implementation of this study. This protocol has been

⁷ Species collection numbers are goals only. Actual numbers collected per developmental window will depend on species availability and numbers of target organisms at each site. More than one collection event per location is planned to try to maximize sample numbers.

reviewed and approved by the EPA, and is attached as **Appendix A.2** to this SAP/QAPP. Protocols in **Appendix A** may change if Remedium selects different asbestos analytical laboratories, in accordance with Section 2.5, *Laboratory Support*, of this SAP/QAPP, and with the requirements specified in **Appendix G**. This protocol will be revised by Remedium (or their contractors) if a different asbestos analytical laboratory is selected in the future.

4.3 Media Sample Collection

Detailed surface water and sediment collection procedures are provided in the study protocol (see **Appendix A.2**). The sample collection procedures are summarized below.

Sediment Collection Prior to Study

As discussed above, prior to use as a reference location, sediment samples will be collected from each candidate reference pond for analysis of asbestos and non-asbestos analytes to ensure that the ponds are not contaminated. Sediment samples will also be collected from the OU3 ponds concomitant with the candidate reference pond sampling for analysis of asbestos and non-asbestos analytes to provide a temporally concurrent data set for comparison to OU3 ponds. Sediment sampling will be performed in basic accordance with the OU3-specific SOP No. 5, *Sediment Sampling* (see **Appendix B**). In brief, surficial sediment will be collected from the pond edge at multiple points around the pond and composited into a single sample. Sediment samples submitted for analysis of LA by PLM (see Section 9.2) and for analysis of non-asbestos analytes (see Section 9.3).

Media Collection During the Study

Once the reference ponds are selected and the study commences, surface water samples will be collected from each OU3 pond throughout the study duration on a weekly basis. Surficial sediment will be collected from each OU3 pond, once at the beginning of the study and once at the end of the study. Surface water and surficial sediment will be collected from each reference pond, once at the beginning of the study and once at the end of the study.

Surface water sampling will be performed in basic accordance with the OU3-specific SOP No. 3, *Surface Water Sampling* (see **Appendix B**), using the direct sampling methods. Surface water samples will be collected using a 500-milliliter (mL) high-density polyethylene (HDPE) wide-mouth bottle. Each surface water sample will have a volume of about 400 mL, to ensure there is ample room at the top of the bottle for ozone treatment. For convenience, surface water bottles should be pre-marked on the outside with permanent marker to indicate the 400 mL fill level. Water samples will be submitted for analysis of total LA by transmission electron microscopy (TEM) as described in Section 9.1.

Sediment sampling will be performed in basic accordance with the OU3-specific SOP No. 5, *Sediment Sampling* (see **Appendix B**). At each sampling location, a multi-point composite sample of surface sediment will be collected. Sediment for asbestos analysis will be collected using the same 500-mL HDPE wide-mouth bottles as utilized for surface water collection, and submitted for analysis of LA by PLM as described in Section 9.2. Sediment for non-asbestos analysis will be submitted in four 4-ounce glass jars (see Section 9.3).

4.4 Field Quality Control Samples

4.4.1 Surface Water

Two types of field QC samples will be collected as part of the surface water sampling – field blanks and field duplicates.

Field Blanks

Field blanks for water are prepared by carrying empty bottles into the field and filling them in the field with water from an uncontaminated source (e.g., store-bought drinking water). Field blanks are collected to evaluate potential contamination during sample collection, shipping and handling, and analysis. For this study, field blanks will be collected at a frequency of 5% (one field blank per 20 field samples). It is the responsibility of the field team leader (FTL) to ensure that the appropriate number of field blanks is collected. At the time of field sample collection, 400 mL of clean water will be placed in the 500-mL HDPE bottles and submitted for analysis along with the accompanying field samples. Field blanks will be given a unique sample number and will be specified as a field blank on the field sample data sheet (FSDS). The field blanks will be analyzed for asbestos fibers by the same method as will be used for field sample analysis. Field blanks will be blind to the laboratory (i.e., the laboratory will not be able to distinguish between field samples and field blanks).

If any asbestos structures are observed on a field blank, the FTL and/or laboratory manager will be notified and will take appropriate measures to ensure staff are employing proper sample handling techniques. In addition, a qualifier of “FB” will be added to the related field sample results in the project database to denote that the associated field blank had asbestos structures detected.

Field Duplicates

Field duplicates for water are a second 400-mL water sample collected sequentially from the same station as the parent sample. The field duplicate is collected using the same collection technique as the parent sample. Water field duplicate samples will be collected at a rate of 1 field duplicate per 20 field samples (5%). It is the responsibility of the FTL to ensure that the appropriate number of field duplicates is collected. Each field duplicate is given unique sample

number, and field personnel record the sample number of the associated co-located sample in the parent sample number field of the FSDS. The same station location is assigned to the field duplicate sample as the parent field sample. Field duplicates will be sent for analysis by the same method as field samples and are blind to the analytical laboratories (i.e., the laboratory cannot distinguish between field samples and field duplicates).

Field duplicate results will be compared to the original parent field sample using the Poisson ratio test using a 90% confidence interval (Nelson 1982). Because field duplicate samples are expected to have inherent variability that is random and may be either small or large, typically, there is no quantitative requirement for the agreement of field duplicates. Rather, results are used to determine the magnitude of this variability to evaluate data usability.

4.4.2 Sediment

Field duplicate samples will be collected as part of the sediment sampling for this study. Field duplicates for sediment are collected from the same area as the parent sample but from different individual sampling points. These samples are collected independent of the original field sample with separate sampling equipment and submitted for analysis along with the collected field samples. The field duplicate contains the same number of subsamples as the parent sample (i.e., if the parent sample is a 5-point composite, the field duplicate sample is also a 5-point composite).

A total of two sediment field duplicate samples will be collected – one at the beginning of the study and one at the end of the study. It is the responsibility of the FTL to ensure that the appropriate number of field duplicates is collected. Each field duplicate is given a unique sample number, and field personnel record the sample number of the associated co-located sample in the parent sample number field of the FSDS. The same station location is assigned to the field duplicate sample as the parent field sample. Field duplicates will be sent for analysis by the same method as field samples and are blind to the laboratories (i.e., the laboratory cannot distinguish between field samples and field duplicates).

Field duplicate results analyzed by PLM will be considered concordant if the reported semi-quantitative bin result for the field duplicate is within one bin of the original parent field sample. The variability between the field duplicate and the associated parent field sample reflects the combined variation in sample heterogeneity and the variation due to measurement error. Because field duplicate samples are expected to have inherent variability that is random and may be either small or large, typically, there is no quantitative requirement for the agreement of field duplicates. Rather, results are used to determine the magnitude of this variability to evaluate data usability.

5.0 In-Stream Caged Fish Studies

5.1 Data Quality Objectives

The following sections implement the seven-step DQO process associated with studies of effects in fish exposed to site waters.

5.1.1 Step 1: State the Problem

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including surface water in LRC. Fish in LRC may be exposed to LA by direct contact with the contaminated surface water. As part of the RI at OU3, the EPA is pursuing multiple lines of evidence to help evaluate if exposure of fish to LA in presents an unacceptable ecological risk.

The first line of evidence that the EPA has investigated consisted of demographic studies of fish density in LRC compared to that in appropriate reference streams. This line of evidence suggests that the density of trout in LRC is lower than expected by comparison to URC and other nearby reference streams (NSY, BTT), and that this difference is statistically significant (Parametrix 2009a, 2010).

Efforts to determine whether these population differences are attributable to habitat differences or to an adverse effect of LA have not been definitive (Parametrix 2009a). Hence, additional information is needed to determine if the apparent reduction in fish population in LRC is likely related to LA or whether the reduction is best understood in terms of habitat.

A second line of evidence that the EPA investigated with the goal of resolving this question consisted of laboratory-based studies in which fish (rainbow trout fry) were exposed to LA in water derived from the site (Parametrix 2009b). Although no adverse effects were observed, it was subsequently determined that LA in the water became clumped by organic material in the water, and these clumps adhered to the walls of the exposure aquaria, reducing exposure concentrations to low levels. Subsequent attempts to solve this problem have not been successful. Consequently, this line of evidence does not provide meaningful results regarding the potential toxicity of LA on fish.

In the absence of the ability to reliably expose fish to LA under laboratory conditions, the OU3 BTAG determined that exposure of fish *in situ* to natural waters of LRC is an appropriate alternative line of evidence to help evaluate risks to fish. The chief advantage of this line of evidence is that the exposure water will reflect actual site conditions. The chief disadvantage is that it is not possible to control concentration levels during the study, so it is unlikely that a dose-response relationship can be established. This weakens the ability to assign causality to

any observed responses. To help strengthen this line of evidence, an additional line of evidence will be gathered as described in Section 6.

5.1.2 Step 2: Identify the Goal of the Study

The goal of this study is to expose fish to site waters in LRC and to determine if the exposure causes an unacceptable increase in the rate of adverse effects compared to that observed in one or more reference streams.

Because the concentration of LA in LRC site water cannot be controlled, and because the concentration of LA has been shown to be quite variable, an important goal of the study is to perform the exposures at a time when exposure levels are at the high end of the concentrations that occur naturally.

In addition, because it is possible that different life stages may have varying susceptibility to LA exposures, another goal of the study is to evaluate any adverse effects for a range of life stages.

5.1.3 Step 3: Identify the Types of Data That Will Be Collected

The data needed to constitute a useful additional line of evidence include the following types of measurements:

- Measures of the frequency or severity of potential adverse effects in several different life stages of an appropriate species of fish exposed in LRC and in one or more appropriate reference stream locations.
- Measurements of the concentration of LA in surface water and in the cages (if different) during the exposure interval.
- Measurements of water temperature in exposure reaches. These data are needed to adjust for the effects of differing temperatures on development and growth rates.

5.1.4 Step 4: Define Study Boundaries

Spatial Bounds

Three streams exist in OU3 that may be impacted by LA: Carney Creek, Fleetwood Creek, and LRC (see **Figure 3-1**). Of these, the best and most extensive fish habitat is present in LRC, and fish population data have been collected for this reach. In addition, concentrations of LA in LRC tend to be the same or higher than in Carney Creek or Fleetwood Creek. Consequently, LRC is the optimum location for fish exposure studies.

Three streams are potentially useful as reference locations: URC, NSY, and BTT. Of these three alternatives, URC (see **Figure 3-1**) is considered to be the best match in terms of water temperature and/or gradient to the upper portion of LRC (i.e., LRC-2), while BTT (see **Figure 4-1**) is considered to be the best match in terms of water temperature and/or gradient to the lower portion of LRC (i.e., LRC-5). Therefore, these two reaches should be used for collection of reference data. However, the selected reference locations may be modified depending upon site conditions at the time of the study.

Temporal Bounds

The concentration of LA in site surface water has been studied at several onsite stations, and substantial variability has been noted. However, in general, the highest concentrations in LRC tend to occur at the time of spring runoff (typically from about mid-April through late June), with peak concentrations in mid-May (see **Figure 5-1**). Because it is important to achieve a high level of exposure in these studies, to the extent possible, all exposures should occur within this spring runoff period.

5.1.5 Step 5: Measurement Endpoints and Data Interpretation

The most appropriate measurement endpoints depend on the life stage selected for evaluation. In general, it is expected that fish are likely most sensitive to environmental toxicants during early life stages. Therefore, the study should include the following two life stages:

- Eyed eggs
- Fry or small juveniles

The following table summarizes the measurement endpoints for each of these life stages:

Life Stage	Measurement Endpoint
Eyed eggs	<ul style="list-style-type: none"> • Hatching success • Post-hatch survival • Overall survival • Size (body weight) • Gross external lesions • General swimming behavior • Histological lesions (optional)
Fry or juvenile	<ul style="list-style-type: none"> • Survival • Growth (body weight gain) • Gross external lesions • General swimming behavior • Histological lesions (optional)

The precise statistical tests that will be used to compare exposed and control organisms will vary between the measurement endpoints. For discrete endpoints (e.g., survival, lesion frequency), it is expected that comparisons will be made using the Fisher Exact test. For continuous endpoints (e.g., body weight), it is expected that the comparisons between control and treated groups will be performed using the WRS Test (unless the data are distributed approximately normally, in which case comparisons may be performed using t-statistics). Other statistical tests that may be appropriate include one-way ANOVA or an ANOVA on ranks. *Post hoc* tests may also be used such as Dunnett's test or Bonferroni t-test for parametric sets, or Dunn's test for non-parametric tests.

If no statistically significant differences in any of the endpoints are detected between the exposed and the reference organisms, it will be concluded that exposure to LA and other site-related contaminants in LRC at concentrations equal to or less than the average level in the water during the exposure period are not likely to cause effects that impair growth, reproduction, or survival. If statistically significant changes in one or more measurement endpoints are observed, additional investigation may be needed to determine if those effects result in ecologically significant effects at the population level (e.g., decreased population density), and if so, to identify the causal agent(s) and to identify a no-effect level and potentially a low-effect level (depending upon the degree of effect severity observed) that may be used to evaluate remedial alternatives. However, the potential need for additional studies will depend in part on the results from other lines of evidence being collected to evaluate risks to fish (see Section 6).

5.1.6 Step 6: Specify Performance or Acceptance Criteria

In evaluating the results of these studies of potential effects on caged fish, two types of decision errors are possible:

- A *false negative decision error* occurs when it is decided that there are no significant effects on fish, when in fact there are.
- A *false positive decision error* occurs when it is decided that there are significant effects on fish, when in fact there are not.

The probability of decision errors when comparing two data sets (site *vs.* reference) is controlled by the selection of the null hypothesis, and by selection of an appropriate statistical method to test the null hypothesis (EPA 2002). Two alternative forms of null hypothesis are possible:

- Form 1: The null hypothesis is that no difference exists between site and reference. A confidence level of $100(1-\alpha)\%$ is required before the null hypothesis is rejected and it can be declared that the site data are higher than the reference data.

- **Form 2:** The null hypothesis is that the site is higher than reference by some amount (S) that is considered to be biologically significant. A confidence level of $100(1 - \alpha)\%$ is required before the null hypothesis is rejected and it is declared that the difference between site and reference, if any, is smaller than S.

For the purpose of this effort, the Form 1 null hypothesis is selected for use, because it is the most familiar, is the easiest to interpret, and does not require specification of an effect that is presumed to be significant. In accordance with EPA (2002), when the Form 1 null hypothesis is used, it is appropriate to select a value of α that is somewhat higher than the usual value of 0.05, such that marginal differences between site and reference are more easily identified as being significant. Therefore, α is set to 0.20.

5.1.7 Step 7: Optimize the Study Design

Based on the DQOs presented above, the following are key study design features of the in-stream caged fish studies.

Exposure Species

Fish population studies indicate that the predominant fish species in LRC is rainbow trout. However, the Montana Department of Fish, Wildlife and Parks (MFWP) has requested that only native cutthroat trout be used in this study to help minimize the chance of releases of (non-native) rainbow trout. On this basis, both the eyed egg study and the caged fish study will utilize only disease-free native cutthroat trout obtained from suitable MFWP fish hatchery stocks.

Reference Locations

During a site visit in late April 2012, the field teams identified several potential issues with the BTT station that could impact the success of the caged studies. The substrate at BTT had a high percentage of clay, which could potentially smother the trout eggs in the creek sediment (even after the addition of other gravel and cobble). In addition, the BTT property owners have the ability to alter the flow to the creek (both reducing flows to maintain water levels in the Bobtail ponds for their cattle and rapidly releasing large volumes of water which could scour the creek and wash out pools). Due to these concerns, BTT will not be utilized as a reference location. Thus, the two reference areas selected for evaluation in both studies will be NSY and UCR.

Eyed Egg Study

Exposure Boxes

Eyed eggs will be placed in Whitlock-Vibert boxes or equivalent (30 eggs per box). After the eggs hatch and after some of the yolk sac has been absorbed, the larval fish fall from the upper egg chamber into a lower protected “nursery” chamber where they develop to the swim-up stage (yolk resorbed). The box shall be enclosed in a mesh to prevent the escape of the swim-ups and to provide protection from predators.

Number and Location of Boxes

A total of six exposure boxes will be placed in LRC. If possible, three will be placed in the general vicinity of LRC-2, and three will be placed in the general vicinity of LRC-5 (see **Figure 3-1**). If suitable locations cannot be located for three boxes at each station, then LRC (between LRC-2 and LRC-5) will be divided into three segments (top, middle, bottom), and two boxes will be located in each segment. A total of three boxes will be placed in URC (see **Figure 3-1**), and three boxes in NSY (see **Figure 4-1**), to serve as references.

Statistical tests will initially be performed on a station by station basis (e.g., LRC-2 *vs.* URC, etc). If the data indicate there are no important differences (e.g., URC is similar to NSY), then the data may be combined across stations to improve statistical power and confidence.

To the extent possible, each exposure location will be selected to resemble a location where a natural redd might occur. The boxes are placed in spawning-size gravel at the selected exposure locations. The top of the box should be several inches below the surface of the gravel. If gravel deposits of suitable particle size are limited or do not exist at the selected exposure location, gravel of the correct size from other nearby locations or from commercial sources may be brought in to cover the boxes.

Timing and Duration of Exposure

Cutthroat trout in Montana mountain streams normally begin to spawn about mid-April, and eyed eggs first become available in State hatcheries about May 1. Eyed eggs should be obtained from the hatchery as soon as possible in this time frame, and placed in LRC and the two reference streams immediately thereafter. This is expected to be during the rising limb of the spring runoff (i.e., in early May, see **Figure 5-1**).

The duration of exposure should be sufficient to allow the eyed eggs to develop through “button-up” (loss of the yolk sack) and into the early days of swim-up. The time required for this developmental transition is dependent upon water temperature. Assuming temperatures in the range of 5 to 10 degrees Celsius over most of the exposure interval, it is expected that the

time to swim-up may range from about 60 to 90 days. Actual time to reach swim-up will be determined by reach-specific temperature measurements as well as direct observations of the organisms.

Evaluation of Endpoints

Measurement endpoints will be evaluated in a laboratory trailer located near OU3. At the end of exposure, the cages will be retrieved and transported in site water to the laboratory where the endpoints will be measured. Swimming behavior will be observed after transferring the living alevins into aquaria containing site water, followed by observation for 30 minutes. Then, the fish will be sacrificed, weight and length measurements made, and individual fish placed in preservative for transport to a histological laboratory for external examination. Internal histology will only be conducted if recommended by the histologist.

Exposure Characterization for LRC Locations

Eggs and pre-swim-up alevins reside in the stream gravel, so the exposure medium of chief concern is the gravel pore water. In order to characterize the concentration of LA in pore water, a sample of water will be removed from one box (selected at random) per station (or per segment), twice per week (e.g., Monday and Thursday) during the exposure interval. Assuming an exposure duration of at least 8 weeks⁸, this will generate at least 16 samples per station (or segment). If the boxes are located in two stations, this will result in at least 32 total samples. If the boxes are distributed across three segments, this will result in a total of at least 48 samples.

Although not expected, there is some possibility that the exposure concentration of LA in the Whitlock-Vibert boxes might be different from that in the overlying stream water, or in the adjacent pore water in the gravel. If so, it is important to characterize these differences so that future monitoring of stream water can be used to evaluate risks to eggs and larva in the gravel. For this reason, samples of pore water in the gravel outside the Whitlock-Vibert boxes and samples of surface water in the overlying stream should be collected from stations in LRC to compare to the sample of water collected from inside the box. Because of the time variability in water concentrations, the pore water samples and overlying water samples from outside the box should be collected at approximately the same time as the corresponding pore water sample from inside the box (e.g., within 10 to 20 minutes of each other). A total of 6 such triplet sets of water samples should be collected (e.g., one triplicate set from each of three locations on each of two separate sampling days). The days on which sampling occurs is not critical.

⁸ The actual number of water samples depends on the number of weeks of the study, which cannot be known with certainty since this depends on creek temperature.

Exposure Characterization for Reference Locations

Pore water samples from within the Whitlock-Vibert boxes should also be collected once per week from one box (selected at random) in URC and NSY. This will result in a total of 16 water samples from the reference locations. Samples from the overlying stream water outside the boxes are not required for the reference locations.

Water Sample Analysis

All water samples from site and reference locations shall be analyzed for total LA using TEM, treating the water with ozone/ultraviolet prior to analysis (see Section 9.1).

Temperature Monitoring

Surface water temperature should be monitored continuously at each eyed egg station (or segment), and the data retrieved no less than once per week.

Juvenile Trout Study

Exposure of caged trout to site and reference waters will be performed in general accordance with the methods that have been developed and used by the MFWP.

Fish Size

MFWP recommends that caged fish studies be performed using fish no smaller than 40 to 60 millimeters (mm). For cutthroat trout, fry are expected to reach this size about August 1, well past the high concentration levels generally associated with spring runoff. Consequently, rather than exposing fry in August, the smallest available cutthroat trout from the prior year's hatch (probably in the 3-6 inch size range) will be obtained from the State hatchery, and these fish will be exposed during spring runoff. This will help ensure that exposure levels are at the high end of the naturally occurring range. In addition, even though cutthroat fry are not expected to be present at this time, the observations may serve as a surrogate for rainbow trout which spawn earlier than cutthroat, and which may have swim-up fry emerge during the falling limb of the spring hydrograph.

Timing and Length of Exposure

Fish shall be placed into the streams about May 15, which typically corresponds to the peak of the spring runoff (based on previous flow data collected in LRC). Placing cages in the stream earlier in the hydrograph is not preferred because this would increase the chances that a cage will be damaged or lost as flows increase. If cages are successfully located at peak flow, then it is expected they will remain stable as flow decreases.

MFWP has demonstrated that in-stream exposures can be performed up to 45 days without excessive mortality. For the purposes of the OU3 study, an exposure duration of about 30 days is considered to be sufficient.

Cage Design and Placement

Similar to the eyed egg study, a total of six cages will be placed in LRC. If possible, three will be placed in the general vicinity of LRC-2, and three will be placed in the general vicinity of LRC-5 (see **Figure 3-1**). If suitable locations cannot be located for three cages at each station, then LRC (between LRC-2 and LRC-5) will be divided into three segments (top, middle, bottom), and two cages will be located in each segment. A total of three cages will be placed in URC (see **Figure 3-1**), and three cages in NSY (see **Figure 4-1**), to serve as reference.

Depending on the size of the fish which are available from the hatchery, there will be about 15 fish per cage. The cages are about 0.04 cubic meters in size (about 13 inches x 15 inches x 13 inches), and are anchored into the stream bottom at each exposure location. The frames are constructed of wood, with 1/4-inch metal mesh on four sides and the bottom which allows water and food to pass through. The top is a solid wood door with attached floats, which provides protection from the sun and most predators.

Cages should be placed in areas where water velocity is <0.75 feet per second (ft/s) (e.g., in an eddy behind a boulder or log). If needed, an artificial barrier may be constructed to create a protective eddy. Cage locations need to be monitored daily to ensure conditions have not changed (e.g., increased flow, debris on cage).

All cages will be provided with an adequate amount of food per day. Cages need to be cleaned periodically (e.g., daily brushing of the mesh). Expired fish will be removed and placed in preservative on a daily basis.

Exposure Characterization for LRC Locations

Caged fish are exposed to LA primarily through surface water. In order to characterize the concentration of LA in surface water in LRC, a sample of water will be removed from one cage per station or per segment, twice per week (e.g., Monday and Thursday) during the exposure interval. Assuming an exposure duration of about 4 weeks, this will generate 8 samples per LRC station (or segment). If the cages are located in two stations, this will result in 16 total samples. If the cages are distributed across three segments, this will result in a total of 24 samples.

Exposure Characterization for Reference Locations

For each of the two reference streams (URC and NSY), one sample of surface water will be removed from one cage (selected at random) once per week. Assuming an exposure duration of 4 weeks, this will result in 8 total samples.

Temperature Monitoring

Surface water temperature should be monitored continuously at each station (or segment) where fry cages are positioned, and the data retrieved no less than once per week. Note: If the fry cage placement locations coincide with the eyed egg cage locations, there is no need to place any additional temperature loggers to monitor surface water temperatures for the fry study.

5.2 Study Protocol

A detailed study protocol for the eyed egg study and the fry study has been developed by Remedium-contracted scientists responsible for field implementation of this study. This protocol has been reviewed and approved by the EPA, and is attached as **Appendix A.3** to this SAP/QAPP. Protocols in **Appendix A** may change if Remedium selects different asbestos analytical laboratories, in accordance with Section 2.5, *Laboratory Support*, of this SAP/QAPP, and with the requirements specified in **Appendix G**. This protocol will be revised by Remedium (or their contractors) if a different asbestos analytical laboratory is selected in the future.

5.3 Media Sample Collection

As discussed above, a series of water samples (both from within the exposure chambers and in the overlying water) are needed to characterize the exposure levels that occurred during the course of each study. The means for collecting these samples from within the chambers are detailed in the study protocol (see **Appendix A.3**).

All water samples will be collected using a 500-mL HDPE wide-mouth bottle. To ensure an adequate volume of water for analysis, each water sample should be no less than 200 mL, with a target volume of about 400 mL. Volumes greater than 400 mL should not be collected to ensure there is ample room at the top of the bottle (headspace) to accommodate the ozone treatment performed by the analytical laboratory. For convenience, these bottles should be pre-marked on the outside with permanent marker to indicate the 200 mL and 400 mL fill levels. These water samples will be submitted for analysis of total LA by TEM as described in Section 9.1.

5.4 Field Quality Control Samples

Two types of field QC samples will be collected as part of the water sampling – field blanks and field duplicates.

Field Blanks

In brief, field blank samples of water (i.e., 400 mL of clean water) should be prepared and submitted in 500-mL HDPE bottles along with the field samples. For this investigation, field blanks will be collected once at the beginning and once at the end of each study (i.e., two field blanks from the eyed egg study and two field blanks from the juvenile fish study). It is the responsibility of the FTL to ensure that the appropriate number of field blanks is collected. At the time of field sample collection, 400 mL of clean water will be placed in the 500-mL HDPE bottles and submitted for analysis along with the accompanying field samples. Field blanks will be given a unique sample number and will be specified as a field blank on the FSDS. The field blanks will be analyzed for asbestos fibers by the same method as will be used for field sample analysis. Field blanks will be blind to the laboratory (i.e., the laboratory will not be able to distinguish between field samples and field blanks).

If any asbestos structures are observed on a field blank, the FTL and/or laboratory manager will be notified and will take appropriate measures to ensure staff are employing proper sample handling techniques. In addition, a qualifier of “FB” will be added to the related field sample results in the project database to denote that the associated field blank had asbestos structures detected.

Field Duplicates

Field duplicates for water are a second 400-mL water sample collected sequentially from the same station as the parent sample. The field duplicate is collected using the same collection technique as the parent sample. For this investigation, field duplicate samples will be collected once at the beginning and once at the end of each study (i.e., two field duplicates from the eyed egg study and two field duplicates from the juvenile fish study). It is the responsibility of the FTL to ensure that the appropriate number of field duplicates is collected. Each field duplicate is given unique sample number, and field personnel record the sample number of the associated co-located sample in the parent sample number field of the FSDS. The same station location is assigned to the field duplicate sample as the parent field sample. Field duplicates will be sent for analysis by the same method as field samples and are blind to the analytical laboratories (i.e., the laboratory cannot distinguish between field samples and field duplicates).

Field duplicate results will be compared to the original parent field sample using the Poisson ratio test using a 90% confidence interval (Nelson 1982). Because field duplicate samples are expected to have inherent variability that is random and may be either small or large, typically,

there is no quantitative requirement for the agreement of field duplicates. Rather, results are used to determine the magnitude of this variability to evaluate data usability.

6.0 Resident Fish Lesion Study

6.1 Data Quality Objectives

The following sections implement the seven-step DQO process associated with a study of potential effects in resident fish exposed to site waters.

6.1.1 Step 1: State the Problem

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including surface water and sediment in LRC. Resident fish in LRC may be exposed to LA by direct contact with the contaminated surface water or sediment. As part of the RI at OU3, the EPA is pursuing multiple lines of evidence to help evaluate if exposure of fish to LA in LRC presents an unacceptable ecological risk.

The first line of evidence that the EPA has investigated consisted of demographic studies of fish density in LRC compared to that in appropriate reference streams. This line of evidence suggests that the density of trout in LRC is lower than expected by comparison to URC and other nearby reference streams (NSY, BTT), and that this difference is statistically significant (Parametrix 2009a, 2010). In particular, the density of small fish (<65 mm), which are usually considered to be young of the year, appears to be much lower in LRC than in reference streams. Efforts to determine whether these differences are attributable to habitat differences or to an adverse effect of LA have not been definitive (Parametrix 2009a). Hence, additional information is needed to determine if the apparent reduction in fish population in LRC may be related to LA exposure or whether the reduction is best understood in terms of habitat.

A second line of evidence that the EPA investigated with the goal of resolving this question consisted of laboratory-based studies in which fish (rainbow trout fry) were exposed to LA in water derived from the site (Parametrix 2009b). Although no adverse effects were observed, it was subsequently determined that LA in the water became clumped by organic material in the water, and these clumps bound to the walls of the exposure aquaria, reducing exposure concentrations to low levels. Subsequent attempts to solve this problem have not been successful. Consequently, this line of evidence does not provide meaningful results regarding the potential toxicity of LA on fish. As an alternative to laboratory exposures, *in situ* exposures of caged fish will be implemented in 2012 (see Section 5).

In the absence of the ability to reliably expose fish to LA under laboratory conditions and because of the inherent limitations of *in situ* exposures, the OU3 BTAG determined that examination of resident fish captured from LRC for the occurrence of external lesions (e.g., gill, lateral line, skin) and possibly internal lesions and/or histological abnormalities (if needed) is an appropriate and potentially useful additional line of evidence to help with the assessment of

risks to fish. This line of evidence can be used to provide information on potential effects in fish that are exposed to LA in site waters, as well as to provide data that may be used to support a causality assessment of effects that may be observed in the caged fish studies (see Section 5).

6.1.2 Step 2: Identify the Goal of the Study

The goal of this study is determine if the frequency of lesions or abnormalities is higher in two size classes of fish from LRC than in similarly sized fish from reference streams.

6.1.3 Step 3: Identify the Types of Data That Will Be Collected

The data needed for this line of evidence include measurement of the frequency of lesions in fish captured from LRC compared with that for fish of comparable size from one or more reference streams.

6.1.4 Step 4: Define Study Boundaries

Spatial Bounds

Onsite Sampling Locations

Three streams exist in OU3 that may be impacted by LA: Carney Creek, Fleetwood Creek, and LRC. Of these, the best and most extensive fish habitat is present in LRC, and fish population data have been collected for this reach. In addition, concentrations of LA in LRC tend to be the same or higher than in Carney Creek or Fleetwood Creek. Consequently, LRC is the optimum location for collection of resident fish that have been exposed to LA.

Within LRC, capture should occur at multiple stations, including the locations where previous population studies have been performed (TPTOE-2, LRC-1, LRC-2, LRC-3, and LRC-5) (see **Figure 3-1**). To the extent feasible, approximately equal numbers of fish should be collected at each station to help ensure the data set is representative and is not unduly influenced by any individual station. To this end, the length of each sampling station may be increased as needed to increase the likelihood of capture.

Reference Sampling Locations

Three streams are potentially useful as reference locations: URC, NSY, and BTT. In selecting which of these is/are most appropriate for use in this study, matching on species of fish present is thought to be most important. Population studies that have been performed to date indicate that fish in LRC consist of rainbow, cutthroat, and cutbow (hybrid) trout. URC contains mainly cutthroat trout, NSY contains mainly cutthroat and cutbow trout, and BTT contains predominantly brook trout. On this basis, BTT is not considered optimal for use as reference

because of potential species differences, but both URC and NSY are potentially valuable and both of these two reaches should be used for collection of reference data.

As above, the capture locations in URC and NSY will be similar to previous study locations (see **Figure 3-1** and **Figure 4-1**, respectively), adjusting the length of the sampling reaches as needed to increase the likelihood of capture.

Target Fish Size and Temporal Bounds on Fish Collection

At present, there are no data to indicate whether there is any important age-dependence in the potential effects of LA on fish. However, young fish are often more sensitive than older fish, at least for some metals. For these reasons, the study should focus on young fish. In general, fish that are <65 mm are considered to be young of year (YOY), although some YOY may grow larger than this by the end of the summer (depending on water temperature and other habitat factors). For this reason, sampling should include all fish that are 100 mm or less in length. This should include all YOY, and will also include some juvenile fish from the previous year's hatch.

The time of year that resident YOY reach a size of about 65 mm depends on species and water temperature. Based on typical growth rates, it is expected that cutthroat trout will reach this size range by about mid-August, and rainbow trout are likely to reach this size somewhat earlier. Based on this, the optimum time for fish collection is early to mid-August.

Target Species

As noted above, population studies that have been performed to date indicate that resident fish in LRC consist of rainbow, cutthroat, and cutbow trout. Therefore, trout that are kept for evaluation in LRC and reference streams should only include these species.

6.1.5 Step 5: Measurement Endpoints and Data Interpretation

All fish will be carefully examined for gross external lesions or abnormalities. The initial examination will be performed under a dissecting light microscope looking for abnormalities, with special attention to gill tissue and lateral line. If tumors or other anomalies are identified, these tumors/abnormalities will be excised, sectioned, stained, and examined microscopically by the pathologist.

All fish collected as part of this study will be maintained by the histology laboratory in the event that more detailed histological preparation and microscopic examination may be needed in the future. For example, if other studies at the site suggest that there are effects on the growth or survival of fish in LRC, then a more detailed analysis for external or internal lesions that might explain these effects may be appropriate.

Lesion data that are recorded by the histologist should include both presence/absence (used to compare frequency) and the degree or severity (minimal, mild, severe) of the lesions. For frequency-based data, it is expected comparisons of site with reference will be performed using the Fisher Exact test. For continuous endpoints (lesion severity), it is expected that the comparisons between control and treated groups will be primarily qualitative, although quantitative tests such as the WRS test may also be useful.

The basic evaluation strategy will be to combine data for all LRC stations and to compare this combined data set to the reference locations, either singly or combined. If the data permit, some comparisons based on stratification by sampling location and/or fish size may also be performed.

If no statistically significant differences in frequency or severity of lesions or abnormalities are detected between exposed (LRC) and reference organisms, this will be interpreted as being consistent with the conclusion that LA and other site-related contaminants in LRC are not likely to cause effects that are ecologically significant. If statistically significant increases in frequency or severity of lesions in fish from LRC are observed, additional investigation may be needed to determine the causal factor(s) (LA *vs.* other site-related contaminants), and to determine if those effects are likely to result in ecologically significant effects at the population level. However, risk management decision-making will utilize information from multiple lines of evidence, so the potential need for additional studies will depend on the results from the other lines of evidence being collected to evaluate risks to fish (see Section 5).

6.1.6 Step 6: Specify Performance or Acceptance Criteria

In evaluating the results of these studies of lesions in fish from LRC, two types of decision errors are possible:

- A *false negative decision error* occurs when it is decided that there are no significant effects on fish, when in fact there are.
- A *false positive decision error* occurs when it is decided that there are significant effects on fish, when in fact there are not.

The probability of decision errors when comparing two data sets (site *vs.* reference) is controlled by the selection of the null hypothesis, and by selection of an appropriate statistical method to test the null hypothesis (EPA 2002). Two alternative forms of null hypothesis are possible:

- Form 1: The null hypothesis is that no difference exists between site and reference. A confidence level of $100(1-\alpha)\%$ is required before the null hypothesis is rejected and it can be declared that the site data are higher than the reference data.

- **Form 2:** The null hypothesis is that the site is higher than reference by some amount (S) that is considered to be biologically significant. A confidence level of $100(1 - \alpha)\%$ is required before the null hypothesis is rejected and it is declared that the difference between site and reference, if any, is smaller than S.

For the purpose of this effort, the Form 1 null hypothesis is selected for use, because it is the most familiar, is the easiest to interpret, and does not require specification of an effect that is presumed to be significant. In accordance with EPA (2002), when the Form 1 null hypothesis is used, it is appropriate to select a value of α that is somewhat higher than the usual value of 0.05, such that marginal differences between site and reference are more easily identified as being significant. Therefore, α is set to 0.20.

6.1.7 Step 7: Optimize the Study Design

Based on the DQOs presented above, the following are key study design features of the fish lesion field study.

Capture methods

Fish will be collected both by electro-shocking and by modified minnow traps. Minnow trap modifications include reducing the size of the opening so only small-sized fish (<100 mm) can enter, and a smaller mesh will be placed over the trap to ensure the targeted fish cannot escape through the sides. Block nets may also be employed if necessary.

Traps will be placed for one-half to one full day (daylight hours only) at each sampling segment within each stream reach (LRC, URC, NSY). If sample numbers are not met in the initial round of trapping, trapping will be repeated for a second round as needed at each location.

Target Sample Size per Site

The minimum number of fish required to identify a statistically significant difference in lesion rate between site and reference fish depends on the actual lesion rates that exist. **Figure 6-1** shows the probability of observing a statistically significant difference using the Fisher Exact test (Form 1 test, $\alpha = 0.2$) as a function of the sample size and the true lesion rate in reference and exposed fish. As seen, the ability to detect a difference of 10-20% in the lesion rate is quite low when sample size is 10 or less, and increases as sample size increases. For this study, a sample size of 20 fish from each sampling reach (LRC, URC, NSY) is identified as the target. If possible, these fish should be approximately evenly distributed between two size classes (<65 mm and 65-100 mm). As noted above, for sampling in LRC, the goal is to collect approximately equal numbers of fish from each of five sampling stations (TPTOE-2, LRC-1, LRC-2, LRC-3 and LRC-5). Therefore the target number in LRC is 4 per station (ideally two of each size class, if possible), with a maximum of 6 per station.

Although a larger sample size would yield increased statistical power, based on historical capture data it is considered likely that collection of a substantially larger number of fish in the targeted size classes may not be feasible. In addition, there may be no clear ability to link lesion rates to population-level impacts, so the observations from this study will not be used in isolation, but only in combination with all other available lines of evidence.

Site Reconnaissance

A creek reconnaissance will be conducted immediately prior to the start of trapping work to establish expanded reach areas and trap placement locations.

6.2 Study Protocol

A detailed study protocol for the fish lesion study has been developed by Remedium-contracted scientists responsible for field implementation of this study. This protocol has been reviewed and approved by the EPA, and is attached as **Appendix A.4** to this SAP/QAPP. Protocols in **Appendix A** may change if Remedium selects different laboratories, in accordance with Section 2.5, *Laboratory Support*, of this SAP/QAPP, and with the criteria and procedures specified in **Appendix G**.

6.3 Media Sample Collection

No samples of surface water are required for this study. This is because the fish collected will have been exposed to site waters for several months or longer, and a single sample collected at the time of fish collection would not necessarily reflect the concentrations to which the fish were exposed. Numerous surface water samples have already been collected from LRC, and additional samples will be collected as part of other studies of this SAP/QAPP. Hence, sufficient data on LA levels in site waters will be available to document the typical levels of exposure of resident fish to LA in site waters.

6.4 Field Quality Control Samples

No field QC samples are required for this study.

7.0 Fish Tissue Burden Assessment

7.1 Data Quality Objectives

The following sections implement the seven-step DQO process associated with the fish tissue burden assessment.

7.1.1 Step 1: State the Problem

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including releases into several streams and ponds within OU3. Although the exposure pathway of primary concern for humans is inhalation of LA, some studies in animals suggest that ingestion of asbestos fibers can result in the growth of benign intestinal polyps (National Toxicology Program [NTP] 1985). There are several potential scenarios where humans could be exposed to LA via ingestion. One such scenario is the ingestion of edible tissue (fillets) from fish that were caught from waters that contain LA. However, at present, there are no data available on the potential concentrations of LA in fish tissue in OU3 or other waters that may contain LA. Thus, data are needed that can be used to determine the potential for uptake of asbestos fiber into fish tissues.

7.1.2 Step 2: Identify the Goal of the Study

The goal of this study is to evaluate the potential for uptake of asbestos fibers into fish tissues.

7.1.3 Step 3: Identify the Types of Data That Will Be Collected

The data that are needed to address the study goal are reliable measures of LA concentrations in tissues of fish exposed to environmental levels of LA that are at the high-end of expected concentrations.

7.1.4 Step 4: Define Study Boundaries

Spatial Bounds

The level of LA in fish tissue is expected to be related to the concentration of LA in water and the length of time the fish have been exposed (i.e., large fish are likely to have higher tissue concentrations than small fish). Thus, to ensure that the evaluation focuses on the high-end of the potential range of exposures, fish should be collected from a location with high LA concentrations and where large fish are present.

Of the waters that support fish and are likely to contain LA, the OU3 on-site tailings impoundment best meets these two requirements: concentrations of LA are relatively high, and large fish are present. Other locations (e.g., LRC, Carney Creek, Fleetwood Creek) have elevated levels of LA, but the sizes of the fish present are relatively small. Fish caught from the Kootenai River below the confluence with Rainy Creek may be large, but concentrations of LA are much lower than in on-site OU3 waters. Therefore, even though access to the tailings impoundment and other OU3 waters is currently restricted to include only authorized personnel, fish should be collected from the tailings impoundment to ensure that fish tissues are at the high-end of the potential range.

Temporal Bounds

Based on the expectation that the concentration of LA in fish tissue will tend to increase over time as a function of the long-term cumulative exposure pattern, it is unlikely that fish tissue burdens will vary substantially due to short-term fluctuations in water concentration. Thus, the timing of the fish collection is primarily based on ease of sample collection. Because fish collection is also planned as part of the *in situ* effects assessment (see Section 6), it is anticipated that sample collection for the tissue burden assessment will occur concomitant with the *in situ* effects assessment sample collection effort.

7.1.5 Step 5: Measurement Endpoints and Data Interpretation

The data from this study will be used to determine the potential for uptake of LA fibers into fish tissues. Remedium is only responsible for the collection of fish; sample preparation and analysis of fish tissue will be performed by EPA contractors as part of a separate study. Thus, the DQOs specified for this study focus only on the sample collection effort and specific measurement endpoints and data interpretation methods for the fish tissue burden assessment are not included in this SAP.

7.1.6 Step 6: Specify Performance or Acceptance Criteria

Because it is not possible at present to quantify the uncertainty in the mean of an asbestos data set as a function of the number of samples, it is not possible to specify a minimum number of samples required to meet performance criteria. In general, more samples are needed when there is high between-sample variability and fewer samples are needed when there is low between-sample variability. In the absence of measured data on LA in fish tissue, this study should seek to collect 5-10 individual fish.

7.1.7 Step 7: Optimize the Study Design

Based on the DQOs presented above, the following are key study design features of sampling effort for the fish tissue burden assessment.

Fish Species

Based on fish population sampling conducted at OU3 in 2008 and 2009, the predominant species in site streams are rainbow trout, cutthroat trout, and rainbow/cutthroat hybrids. Because all these species may be kept for human consumption, any of these species are appropriate for collection as part of this study.

Fish Size

Because it is expected that tissue burdens will increase as a function of fish age, which is directly correlated with size (length), collection efforts will attempt to collect fish that are larger in size, to maximize potential fish tissue burdens. The minimum fish size for this study is about 8 inches. The method of fish collection is less important than ensuring the resulting fish are of adequate size (i.e., angling, netting, and electro-shocking are all viable collection techniques).

7.2 Study Protocol

A detailed study protocol for the collection of fish from OU3 has been developed by the scientists responsible for field implementation of this SAP/QAPP. This protocol has been reviewed and approved by the EPA, and is attached as **Appendix A.5** to this SAP/QAPP.

The preparation of the fish samples and analysis of LA in fish tissues will not be completed under this SAP/QAPP (i.e., Remedium is only responsible for the collection of fish).

7.3 Media Sample Collection

No samples of water are required for this study. This is because the fish collected will have been exposed to site waters for several years or longer, and a single sample collected at the time of fish collection would not necessarily reflect the concentrations to which the fish were exposed. Numerous water samples have already been collected from the site, and additional samples will be collected as part of other components of this SAP/QAPP. Hence, sufficient data on LA levels in site waters will be available to document the typical levels of exposure of resident fish to LA in site waters.

7.4 Field Quality Control Samples

No field QC samples are required for this study.

8.0 Field Procedures and Requirements

8.1 Field Training

Asbestos is a hazardous substance that can increase the risk of cancer and serious non-cancer effects in people who are exposed by inhalation. Therefore, all individuals involved in the collection, packaging, and shipment of samples must have appropriate training. Prior to starting any field work, any new field team member must complete the following, at a minimum:

Training Requirement	Location of Documentation Specifying Training Requirement Completion
Read and understand the governing Health and Safety Plan (HASP)	HASP signature sheet
Attend an orientation session with the field health and safety (H&S) manager	Orientation session attendance sheet
Occupational Safety and Health Administration (OSHA) 40-Hour Hazardous Waste Operations and Emergency Response (HAZWOPER) and relevant 8-hour refreshers	OSHA training certificates
Current 40-hour HAZWOPER medical clearance	Physician letter in the field personnel files
Respiratory protection training, as required by 29 CFR 1910.134	Training certificate
Asbestos awareness training, as required by 29 CFR 1910.1001	Training certificate
Sample collection techniques	Orientation session attendance sheet

It is the responsibility of the field project manager to ensure that all training documentation is up-to-date and on file for each field team member.

A field readiness review meeting will be conducted before beginning field sampling activities, to discuss and clarify the following:

- Objectives and scope of the fieldwork
- Equipment and training needs
- Field operating procedures, schedules of events, and individual assignments
- Required QC measures
- H&S requirements

It is the responsibility of each field team member to review and understand all applicable governing documents associated with this sampling program, including this SAP/QAPP, all associated SOPs (see **Appendix B**), and the applicable HASP. The FTL will oversee all sample collection activities to ensure that governing documents are implemented appropriately.

Field staff that are responsible for the amphibian field study are also required to attend the 3-day training session, which will guide personnel on the procedures for observing, identifying, and collecting amphibian specimens for this study (see **Appendix A.2**).

8.2 Inventory and Procurement of Equipment and Supplies

Prior to initiation of any sampling activities, it is the responsibility of the FTL to review the respective field protocols (see **Appendix A**) and determine the equipment and supplies that are necessary to conduct sampling activities. The FTL will check the field equipment/supply inventory and procure any additional equipment and supplies that are not already contained in the field equipment supply inventory.

The following list summarizes the general equipment and supplies that will be required for most of the studies:

- *Sampling equipment* – See Section 8.5 for sample collection SOPs and medium-specific sampling equipment lists.
- *Field logbook* – Used to document field sampling activities and any problems in sample collection or deviations from this SAP/QAPP. See Section 8.6.3 for standard procedures for field logbooks.
- *FSDS forms* – FSDSs are medium-specific forms that are used to document sample details (i.e., sampling location, sample number, medium, field QC type, etc.). See Section 8.6.2 for standard procedures for the completion of FSDSs.
- *Sample number labels* – Sample numbers for abiotic media (i.e., surface water and sediment) are sequential numbers with investigation-specific prefixes (see Section 8.6.1). Sample number labels are pre-printed and checked out to the field teams by the FTL or their designate. To avoid potential transcription errors in the field, multiple labels of the same sample number are prepared – one label is affixed to the collected sample, one label is affixed to the FSDS. Labels may also be affixed to the field logbook or other field documentation forms. See Section 8.6.1 for standard procedures for the use of sample number labels.
- *Indelible ink pen, permanent marker* – Indelible ink pens are used to complete required manual data entry of information on the FSDS and in the field logbook (pencil may not be used). Permanent markers may be used to write sample numbers on the sample container if pre-printed labels are not available.

- *Personal protective equipment* - As required by the HASP.
- *Digital camera* – Used to document sampling locations and conditions.
- *Global positioning system (GPS) unit, measuring wheel, stakes* – Used to identify and mark sampling locations. See Section 8.7 for standard procedures in GPS documentation.
- *Decontamination equipment* – Used to remove any residual asbestos contamination on reusable sampling equipment between the collection of samples. See Section 8.8 for standard decontamination procedures.

8.3 Inspection/Acceptance of Field Supplies and Consumables

In advance of field activities, the FTL will ensure any in-house measurement and test equipment used to collect data/samples as part of this SAP/QAPP is in good, working order, and any procured equipment is acceptance tested. Any items that the FTL determines are unacceptable will be removed from inventory and repaired or replaced as necessary.

8.4 Field Equipment Maintenance and Calibration

All field equipment should be maintained and calibrated in basic accordance with manufacturer specifications. When a piece of equipment is found to be operating incorrectly, the piece of equipment will be labeled “out of order” and placed in a separate area from the rest of the sampling equipment. The person who identified the equipment as out-of-order will notify the FTL overseeing the investigation activities. It is the responsibility of the FLT to facilitate repair of the out-of-order equipment. This may include having appropriately trained field team members complete the repair or shipping the malfunctioning equipment to the manufacturer. Field team members will have access to basic tools required to make field acceptable repairs. This will ensure timely repair of any out-of-order equipment.

8.5 Sample Collection

Samples of abiotic media (surface water, sediment) and biota (amphibians, fish) will be collected in accordance with the procedures specified in the respective protocols developed by the scientists responsible for field implementation of this SAP/QAPP (see **Appendix A**) and the OU3-specific SOPs (see **Appendix B**).

8.6 Sample Handling and Custody

8.6.1 Sample Identification

For field-collected abiotic media (i.e., surface water and sediment), samples will be labeled with sample numbers supplied by the OU3 data manager (CDM Smith) and will be signed out by the sampling teams. Labels for field-collected surface water and sediment samples will be affixed to the outside of the sample container and covered with a piece of clear packaging tape. Sample numbers will identify the abiotic samples collected during this sampling investigation using the following format:

P5-2####

where:

P5-2 = Prefix that designates samples collected under this Phase V Part B SAP/QAPP

= A sequential four-digit number

The exception to this will be the sediment samples collected for the amphibian toxicity test and the initial sediment collected in support of the amphibian reference pond selection. These samples will be assigned self reading field identifiers (e.g., TP-TOE2_LOT1_REP1). Sample labels for these collection efforts will be supplied by the OU3 data manager (CDM Smith) for use by the sampling teams.

For biota specimens (i.e., fish and amphibians), the respective protocols for each study (see **Appendix A**) provide detailed information for assigning field-generated sample identification (ID) numbers (e.g., TP-PRO-SF-060112-11). Field sample ID numbers for fish and amphibian specimens will be affixed to or written on the outside of the zip-top bag and covered with a piece of clear packaging tape.

8.6.2 Field Documentation

Field teams will record sample information on the most current version of the OU3-specific FSDS for each field-collected surface water and sediment sample (see **Appendix C**) in accordance with the procedures specified in OU3-specific SOP No. 9, *Field Documentation* (see **Appendix B**).

Field documentation for fish and amphibian specimens will be recorded on the field documentation forms developed for each study, as provided in the study protocols (see **Appendix A**).

Scanned copies of all FSDSs and field documentation forms will be posted to the OU3 eRoom on a bi-weekly basis (see Section 10.1.1).

8.6.3 *Field Logbooks*

The field logbook is an accounting of activities at the Site and will duly note problems or deviations from the governing SAP/QAPP or SOPs. Separate field logbooks will be kept for each study and the cover of each field logbook will clearly indicate the name of the associated study. Field logbooks will be completed prior to leaving a sampling location. Field logbooks will be checked for completeness on a daily basis by the FTL or their designate for the first week of each study. When incorrect field logbook completion procedures are discovered during these checks, the errors will be discussed with the author of the entry and corrected. Erroneous information recorded in a field logbook will be corrected with a single line strikeout, initial, and date. The correct information will be entered in close proximity to the erroneous entry. Scanned copies of all field logbooks will be posted to the OU3 eRoom on a bi-weekly basis (see Section 10.1.1).

8.6.4 *Field Sample Custody*

Field sample custody will follow the requirements specified in OU3-specific SOP No. 9 (see **Appendix B**). In brief, all teams will ensure that samples, while in their possession, are maintained in a secure manner to prevent tampering, damage, or loss. All samples and FSDSs will be relinquished by field staff to the field sample coordinator or a designated secure sample storage location at the end of each day.

8.6.5 *Chain-of-Custody Requirements*

The chain-of-custody (COC) record is employed as physical evidence of sample custody and control. This record system provides the means to identify, track, and monitor each individual sample from the point of collection through final data reporting. A completed COC record is required to accompany each shipment of samples. Sample custody will be maintained until final disposition of the samples by the laboratory and acceptance of analytical results by the EPA. **Appendix D** contains the COC form that should be used in this investigation.

The field sample coordinator will prepare a hard copy COC form using the 3-page carbon copy forms developed specifically for use in this investigation. The bottom copy of the COC will be retained by the field sample coordinator and the original COC will accompany the sample shipment.

If any errors are found on a COC after shipment, the hard copy of the COC retained by the field sample coordinator will be corrected and a corrected COC will be provided to the laboratory coordinator (LC) for distribution to the appropriate laboratory.

8.6.6 *Sample Packaging and Shipping*

Samples will be packaged and shipped in basic accordance with the procedures specified in OU3-specific SOP No. 8, *Sample Handling and Shipping* (see **Appendix B**).

Surface water samples for asbestos analysis will be delivered to the selected TEM asbestos analytical laboratory for analysis by TEM (see Section 9.1.1). Unless Remedium identifies a suitable sediment sample preparation laboratory that meets the requirements set forth in **Appendix G**, sediment samples for asbestos analysis will be hand-delivered to the SPF located in Troy, Montana, for preparation and subsequent transfer to the selected PLM asbestos analytical laboratory for analysis by PLM (see Section 9.2). Sediment samples for non-asbestos analysis (see Section 9.3) will be shipped via overnight delivery to Energy Laboratory in Billings, Montana. Fish collected for tissue analysis of asbestos by TEM (see Section 9.1.2) will be hand-delivered to the CDM Smith field office in Libby for subsequent shipment via overnight delivery to the selected TEM analytical laboratory (in coordination with EPA). *[Note: Preparation and analysis of fish tissue samples is not part of this OU3 SAP/QAPP.]* Before sealing the shipping container, the field sample coordinator will sign the COC record and retain the bottom copy of the COC record for the project record.

8.6.7 *Holding Times and Sample Preservation*

A holding time is defined as the allowable time between sample collection and analysis and/or extraction recommended to ensure accuracy and representativeness of analysis results, based on the nature of the analyte of interest and chemical stability factors. The holding time is calculated from the date and time of sample collection to the time of sample preparation and/or analysis. Sample holding times are established to minimize chemical changes in a sample prior to analysis and/or extraction.

Asbestos

In general, there are no holding time requirements for asbestos. Because sample preparation will include techniques to address any issues related to holding time for the medium (e.g., organic material binding asbestos in water), there are no holding time requirements for the surface water, sediment, or fish tissue samples collected as part of this sampling investigation. To limit biological growth, samples should be kept on ice or refrigerated until samples can be prepared by the analytical laboratory.

Non-Asbestos

As noted above, sediment samples collected for the amphibian toxicity test and candidate reference ponds for the amphibian field study will be analyzed for a variety of non-asbestos

analytes (see Section 9.3.2). **Table 8-1** provides the holding time and preservation requirements for sediment for each type of non-asbestos analysis.

Overlying water from the amphibian toxicity test will be analyzed for a variety of water quality parameters (see Section 9.3.1). All water quality analyses of overlying water will be performed in-house by the toxicity testing laboratory (FEL).

8.7 Global Positioning System Coordinate Collection

GPS coordinates will be recorded for each sampling station/reach in basic accordance with the procedures specified in OU3-specific SOP No. 11, *GPS Data Collection* (see **Appendix B**). The following table summarizes the GPS coordinates that will be collected as part of each study:

Study	GPS Coordinate Requirements
Amphibian Toxicity Test	TP-TOE2, CC-1: No GPS coordinates required**
Amphibian Field Study	Site locations: No GPS coordinates required** Reference locations: Single GPS coordinate at the approximate sampling area or pond
In-stream Caged Fish Study	Single GPS coordinate for each cage (or group of cages) within each station (or segment)
Resident Fish Lesion Study	GPS coordinates for the upper and lower boundaries of the sampling reach
Fish Tissue Study	No GPS coordinates required

**Unless sampled locations are different from the stations sampled in previous investigations

If any sampling stations become inaccessible, this information should be documented in the field logbook.

8.8 Equipment Decontamination

Decontamination of non-disposable sampling equipment will be conducted in basic accordance with the procedures specified in OU3-specific SOP No. 7, *Equipment Decontamination* (see **Appendix B**). Materials used in the decontamination process will be disposed of as investigation-derived waste (IDW) as described below.

8.9 Handling Investigation-Derived Waste

Any disposable equipment or other IDW will be handled in basic accordance with the procedures specified in OU3-specific SOP No. 12, *IDW Management* (see **Appendix B**).

8.10 Record of Field Modifications

Minor deviations (i.e., those that will not impact data quality or usability) encountered in day-to-day field work will be noted in the field logbook. Major deviations from this SAP/QAPP that modify the sampling approach and associated guidance documents will be recorded on a field record of modification (ROM) form (see **Appendix E**). Field ROMs will be completed by the FTL, or by assigned field or technical staff. Each completed ROM is assigned a unique number that is specific to each investigation (e.g., Phase V-B LFM-OU3-01) by EPA RPM or their delegate. Once a form is prepared, it is submitted to the EPA RPM for review and approval. Copies of approved field ROMs are available in the OU3 eRoom and are posted to the OU3 website.

9.0 Sample Preparation and Analysis Requirements

Sections 9.1 to 9.3 summarize the analytical requirements for the ecological studies discussed above. **Table 9-1** provides an overview of the samples that will be collected and analyzed for each study. **Appendix F** provides an analytical requirements summary sheet (**OU3VB-0412**), which details the specific analytical requirements for asbestos analyses associated with this sampling investigation. A copy of this summary sheet will be submitted with each COC.

Sections 9.4 to 9.7 summarize the laboratory QA/QC procedures, analytical turn-around times, custody procedures, and data reporting requirements for the analytical laboratories.

9.1 Asbestos Analysis by TEM

9.1.1 Water

The preparation and analysis of all water samples will be performed by the selected TEM analytical laboratory.

Sample Preparation

Water samples shall be prepared for asbestos analysis in basic accordance with the techniques in EPA Method 100.2, as modified by Libby Laboratory Modification⁹ LB-000020A. In brief, water samples will be prepared using an ozone/ultraviolet treatment that oxidizes organic matter that is present in the water or on the walls of the bottle, destroying the material that causes clumping and binding of asbestos structures. Following treatment, an aliquot of water (generally about 5-50 mL, depending on LA concentration) will be filtered through a 25-mm diameter polycarbonate filter with a pore size of 0.1 micrometer (µm) with a mixed cellulose ester filter (0.45 µm pore size) used as a support filter. Approximately one quarter of the filter will be used to prepare a minimum of three grids using the grid preparation techniques described in Section 9.3 of International Organization for Standardization (ISO) 10312:1995(E) (ISO 1995).

Note: If heavy sediment loading is visually noted in the water samples, the preparation procedure may be modified to include a 15-minute settling period to allow the larger particles to fall out of suspension.

⁹ Signed copies of all Libby Laboratory Modifications are available in the Libby Lab eRoom.

Analysis Method and Counting Rules

Grids will be examined by TEM in basic accordance with the procedures described in ISO 10312:1995(E), as modified by the most recent versions of Libby Laboratory Modifications LB-000016, LB-000029, LB-000066¹⁰, LB-000067, and LB-000085.

All structures with fibrous morphology, an x-ray diffraction pattern consistent with amphibole asbestos, a energy dispersive spectrum consistent with LA, length greater than or equal to 0.5 µm, and an aspect ratio (length:width) greater than or equal to 3:1 will be counted and recorded. If observed, chrysotile structures will not be recorded, but the presence of chrysotile structures should be recorded in the analysis comments. Raw structure data (i.e., structure type, length, width, etc.) will be recorded on the OU3-specific bench sheets and electronic data deliverable (EDD) spreadsheet for TEM analyses of water¹¹.

Target Analytical Sensitivity

The target analytical sensitivity (TAS) for water samples analyzed as part of this study is 50,000 L⁻¹. This TAS is consistent with previous surface water sampling efforts conducted at OU3 to date, and is adequate to reliably detect and quantify LA in most water samples.

Maximum Number of LA Structures

For filters that have high asbestos loading, reliable estimates of concentration may be achieved before achieving the TAS. This is because the uncertainty around a TEM estimate of asbestos concentration in a sample is a function of the number of structures observed during the analysis. The confidence interval (CI) around a count of N structures is characterized as a chi-squared (CHISQ) distribution:

$$N_{\text{true}} \sim \frac{1}{2} \cdot \text{CHISQ}(2 \cdot N_{\text{observed}} + 1)$$

As N_{observed} increases, the absolute width of the CI range increases, but the relative uncertainty (expressed as the CI range divided by N_{observed}) decreases. This concept is illustrated in **Figure 9-1**. The goal is to specify a target N such that the resulting Poisson variability is not a substantial factor in the evaluation of method precision. As shown in **Figure 9-1**, above about 25 structures, there is little change in the relative uncertainty. Therefore, the count-based stopping rule for TEM should utilize a maximum structure count of 25 LA structures.

¹⁰ Effective June 13, 2012, all TEM analyses performed for OU3 will utilize Libby Laboratory Modification #LB-000066d (rather than 66c); which effectively decreases the number of micrographs that are taken for each analysis.

¹¹ Copies of the current versions of the OU3-specific EDDs are provided in the OU3 eRoom.

Maximum Area to be Examined

The number of grid openings (GOx) that must be examined to achieve the TAS is calculated as:

$$\text{GOx} = \text{EFA} / (\text{TAS} \cdot \text{Ago} \cdot \text{V})$$

where:

GOx = Number of grid openings

EFA = Effective filter area (assumed to be 1295 square millimeters [mm²])

TAS = Target analytical sensitivity (L⁻¹)

Ago = Grid opening area (assumed to be 0.01 mm²)

V = Water volume applied to the filter (L)

Assuming that 0.1 L of water is able to be applied to the filter, a total of 26 grid openings would need to be examined to achieve the TAS. If less water is filtered, the number of grid openings needed to achieve the TAS increases. In order to limit the level of effort (and cost) for any one analysis, the maximum number of grid openings to be examined for this project is 100 grid openings. Assuming that each grid opening has an area of about 0.01 mm², this would correspond to a maximum area examined of about 1.0 mm².

TEM Stopping Rules

The TEM stopping rules for all surface water samples from this investigation are as follows:

1. Count a minimum of two grid openings from each of two grids.
2. Continue counting until one of the following is achieved:
 - a. The target analytical sensitivity of 50,000 L⁻¹ has been achieved.
 - b. 25 LA structures have been observed.
 - c. A total filter area of 1.0 mm² has been examined (this is approximately 100 grid openings).

When one of these criteria has been satisfied, complete the examination of the final grid opening and stop.

Phased Analysis

The EPA may choose to perform the analysis of water samples using a phased approach. Whether or not the analysis of all samples will be performed will depend upon the magnitude and variability of the analysis results.

9.1.2 Fish Tissue

As noted previously, the preparation and analysis of fish tissue will not be performed under this OU3 SAP/QAPP.

9.2 Asbestos Analysis by PLM

Sample Preparation

Unless Remedium identifies a suitable sediment sample preparation laboratory that meets the requirements set forth in **Appendix G**, all sediment samples collected for asbestos analysis will be transmitted to the Troy SPF for preparation. Samples will be prepared in accordance with SOP ISSI-LIBBY-01. In brief, the raw sediment sample is dried and then split into two aliquots. One aliquot is placed into archive, and the other aliquot is sieved into coarse ($> \frac{1}{4}$ inch) and fine fractions. The fine fraction is ground to reduce particles to a diameter of 250 μm or less and this fine-ground portion is split into 4 aliquots.

Sample Analysis

The PLM analysis of all sediment samples will be performed by the selected PLM asbestos analytical laboratory. Each sediment sample will be analyzed for LA in accordance with Libby site-specific SOPs. The coarse fraction (if any) will be examined using stereomicroscopy, and any particles of LA will be removed and weighed in accordance with SOP SRC-LIBBY-01, referred to as "PLM-Grav." One of the fine-ground fraction aliquots will be analyzed by PLM using the visual area estimation method in accordance with SOP SRC-LIBBY-03, referred to as "PLM-VE." Mass fraction estimates of LA and optical property details will be recorded on the OU3-specific laboratory bench sheets and EDD spreadsheets for PLM.

9.3 Non-asbestos Analytical Methods

9.3.1 Sediment

Prior to use in the amphibian toxicity tests (see Section 3), collected sediment from TP-TOE2 and CC-1 will be analyzed for the following:

- Metals and metalloids – [EPA 6010/6020B]
- Mercury – [EPA 7471A]
- Organochlorine pesticides – [EPA 8081A]
- Chlorinated herbicides – [EPA 8151A]
- Polychlorinated biphenyls – [EPA 8082]
- Semi-volatile organic compounds – [EPA 8270C]

- Diesel/gasoline range organics – [EPA 8015B]
- Acid volatile sulfide – [AVS/TTR]
- Ammonia – [ASAM 33-7]
- Total organic carbon – [ASAM 29-3]
- pH – [ASAM 10-3]
- Moisture – [ASTM D2974]

Analysis for organophosphate pesticides should be performed if available information suggests that such products have been applied in the area of the amphibian reference ponds within the past month.

Before the amphibian field study commences, sediment from each candidate reference pond and each OU3 pond will be analyzed for the above list of analytes prior to the selection of reference areas for the amphibian field study (see Section 4).

All non-asbestos analyses of sediment will be performed by Energy Laboratories.

9.3.2 Surface Water

In the amphibian toxicity tests (see Section 3), the overlying water for each replicate at the beginning and the termination of the toxicity test will be analyzed for the following:

- Total hardness – [EPA 103.2]
- Alkalinity – [EPA 130.1]
- Conductivity – [EPA 120.1]
- Total residual oxidants/residual chlorine – [DPD Method; EPA Method 8167/10070 approved with distillation]
- Ammonia-nitrogen – [Nessler Method; EPA-approved with distillation]

All analyses of overlying water will be performed in-house by the toxicity testing laboratory (FEL).

9.4 Troy SPF QA/QC Procedures

As noted above, all sediment¹² samples submitted for analysis by the Libby-specific PLM methods (i.e., PLM-Grav and PLM-VE) are first processed in accordance with SOP ISSI-LIBBY-01. This processing includes drying, splitting, sieving, grinding, and archiving. Unless Remedium identifies a suitable sediment sample preparation laboratory that meets the

¹² For the purposes of this section, the term “soil” will be used to refer to the preparation of all soil and soil-like (e.g., sediment) materials.

requirements set forth in **Appendix G**, these sample processing activities will be completed at the Troy SPF.

The QA/QC of the soil preparation process is maintained by adherence to standard preparation procedures, submission of preparation QC samples, facilities monitoring, and audits. These procedures and requirements are summarized in below. Detailed information regarding soil preparation procedures and requirements for the Troy SPF can be found in SOP ISSI-LIBBY-01, the *Soil Sample Preparation Work Plan*, and the *ESAT Site Safety Plan*.

9.4.1 Training and Personnel Requirements

Personnel performing sample preparation activities must have read and understood the *Soil Sample Preparation Work Plan*, the *SPF HASP*, and all associated SOPs and governing documents for soil preparation (e.g., SOP ISSI-LIBBY-01). In addition, all personnel must have completed 40-hour OSHA HAZWOPER training, annual updates, annual respirator fit tests, and annual or semi-annual physicals, as required.

Prior to performing activities at the Troy SPF, new personnel will be instructed by an experienced member of the SPF staff and training sessions will be documented in the SPF project files. It is the responsibility of the SPF QAM to ensure that all personnel have completed the required training requirements.

9.4.2 Preparation QC Samples

Four types of preparation QC samples are collected during the soil preparation process – sand blanks, drying blanks, grinding blanks, and preparation duplicates. Each type of preparation QC sample is described in more detail below.

Sand Blank

A sand blank is a sample of store-bought quartz sand that is analyzed to ensure that the quartz sand matrix used for drying and grinding blanks is asbestos-free. Detailed procedures for this certification process are provided in ESAT SOP PLM-02.00, *Blank Sand Certification by PLM*. In brief, about 800 grams of sand are split into 40 sand blank aliquots of roughly equal size. Each sand blank is evaluated using stereomicroscopic examination and analyzed by PLM-VE. If a sand blank has detected asbestos, it is re-analyzed by a second PLM analyst to verify the presence of asbestos. The sand is certified as asbestos-free if all 40 sand blanks are non-detect for asbestos. The sand is rejected for use if any asbestos is detected in the sand blanks. Only sand that has been certified as asbestos-free will be utilized in the SPF.

Drying Blank

A drying blank consists of approximately 100 to 200 grams of asbestos-free quartz sand that is processed with each batch of field samples that are dried together (usually this is approximately 125 samples per batch). The drying blank is then processed identically to field samples. Drying blanks determine if cross-contamination between samples is occurring during sample drying. One drying blank will be processed with each drying batch per oven. It is the responsibility of the SPF QAM to ensure that the appropriate number of drying blanks is collected. Each drying blank is given unique sample number that is investigation-specific, as provided by the field sample coordinator (i.e., a subset of sample numbers for each investigation will be provided for use by the SPF). SPF personnel will record the sample number of the drying blank on the sample drying log sheet.

It is the responsibility of the QATS contractor (or their designate) to review the drying blank results and notify the SPF QAM immediately if drying blank results do not meet acceptance criteria and if corrective actions are necessary. If asbestos is detected by PLM-VE in the drying blank (i.e., result is not Bin A), a qualifier of "DB" will be added to the related field sample results in the project database that were dried at the same time as the detected drying blank to denote that the associated drying blank had detected asbestos. In addition, the drying oven will be thoroughly cleaned. If asbestos continues to be detected in drying blanks after cleaning occurs, sample processing must stop and the drying method and decontamination procedures will be evaluated to rectify any cross-contamination issues.

Grinding Blank

A grinding blank consists of asbestos-free quartz sand and is processed along with the field samples on days that field samples are ground. Grinding blanks determine if decontamination procedures of laboratory soil processing equipment used for sample grinding and splitting are adequate to prevent cross-contamination. Grinding blanks are prepared at a frequency of one per grinding batch per grinder per day. It is the responsibility of the SPF QAM to ensure that the appropriate number of grinding blanks are collected. Each grinding blank is given unique sample number that is investigation-specific, as provided by the field sample coordinator. SPF personnel will record the sample number of the grinding blank on the sample preparation log sheet.

It is the responsibility of the QATS contractor (or their designate) to review the grinding blank results and notify the SPF QAM immediately if drying blank results do not meet acceptance criteria and if corrective actions are necessary. If any asbestos is detected by PLM-VE in the grinding blank (i.e., result is not Bin A), a qualifier of "GB" will be added to the related field sample results in the project database that were ground at the same time as the detected grinding blank to denote that the associated grinding blank had detected asbestos. In addition, the grinder will be thoroughly cleaned. If asbestos continues to be detected in grinding blanks

after cleaning occurs, sample processing must stop and the grinding method and decontamination procedures will be evaluated to rectify any cross-contamination issues.

Preparation Duplicate

Preparation duplicates are splits of field samples submitted for sample preparation. The preparation duplicates are used to evaluate the variability that arises during the soil preparation and analysis steps. After drying, but before sieving, a preparation duplicate is prepared by using a riffle splitter to divide the field sample (after an archive split has been created) into two approximately equal portions, creating a parent and duplicate sample.

Preparation duplicate samples are prepared at a rate of 1 per 20 samples (5%) of OU3 samples prepared. It is the responsibility of the SPF QAM to ensure that the appropriate number of preparation duplicates is prepared. Each preparation duplicate is given unique sample number that is investigation-specific, as provided by the field sample coordinator. SPF personnel will record the sample number of the preparation duplicate and its associated parent field sample on the sample preparation log sheet. Preparation duplicates are submitted blind to the laboratory for analysis by the same analytical method as the parent sample.

Preparation duplicate results will be considered concordant if the reported PLM bin for the preparation duplicate is within one bin of the original parent field sample. The variability between the preparation duplicate and the associated field sample reflects the combined variation due to sample preparation and due to measurement error. Results for preparation duplicate samples are evaluated by the QATS contractor (or their designate). If the concordance rate for preparation duplicate samples is less than 10%, the QATS contractor will notify the SPF QAM to determine if corrective action is needed.

9.4.3 Performance Evaluation Standards

The U.S. Geological Survey (USGS) has prepared several Site-specific reference materials of LA in soil that are utilized as performance evaluation (PE) standards to evaluate PLM-VE laboratory accuracy and precision. These PE standards are kept in storage at the Troy SPF and are inserted into the sample train during soil sample processing. In accordance with SOP ISSI-LIBBY-01, PE standards are inserted both pre- and post-processing. PE standards of varying nominal levels will be inserted quarterly at a rate of at least one per PLM laboratory when soil processing is occurring.

It is the responsibility of the SPF QAM to ensure that the appropriate number of PE standards is inserted. Each PE standard is given unique sample number that is investigation-specific, as provided by the field sample coordinator. SPF personnel will record the sample number of the PE standard, the nominal level of the PE standard, and whether it was inserted pre- or post-

processing on the sample preparation log sheet. PE standards are submitted blind to the laboratory for analysis by the same analytical method as the field samples.

Results for PE standards will be evaluated by the QATS contractor (or their designate). PE standard results are ranked as acceptable if the correct semi-quantitative bin is reported, as determined by the nominal concentration of the PE standard. The LC should be notified if PE standard results do not meet acceptance criteria. Corrective action will be taken if the PE standards demonstrate issues with accuracy and/or bias in PLM-VE results reporting. Examples of corrective actions that may be taken include reanalysis and/or repreparation, collaboration between and among laboratories to address potential differences in analysis methods, and analyst re-training.

9.5 Analytical Laboratory QA/QC Procedures for Asbestos

All laboratories selected for analysis of samples for asbestos will be part of the Libby analytical team. These laboratories have all demonstrated experience and expertise in analysis of LA in environmental media, and all are part of an on-going site-specific QA program designed to ensure accuracy and consistency between laboratories. These asbestos laboratories are audited by the QATS contractor (see Section 11.1.2) and National Voluntary Laboratory Accreditation Program (NVLAP) on a regular basis.

Laboratory QA/QC activities include all processes and procedures that have been designed to ensure that data generated by an analytical laboratory are of high quality and that any problems in sample preparation or analysis that may occur are quickly identified and rectified. Laboratories handling samples collected as part of this sampling investigation will be provided a copy of and will adhere to the requirements of this SAP/QAPP. This section describes the laboratory QA/QC procedures that are required of each laboratory that analyzes field samples from OU3.

9.5.1 Laboratory Quality Assurance Management Plan

Each analytical laboratory has developed a laboratory-specific *QA Management Plan* that provides a detailed description of the procedures and policies that are in place at their laboratory to ensure laboratory quality. This laboratory *QA Management Plan* will include information on standard laboratory methods and SOPs, instrument testing, inspection, maintenance, and calibration requirements, procedures for inspection of supplies and consumables, analyst training, facility contamination monitoring, and internal auditing. These laboratory *QA Management Plans* are reviewed and approved by the LC when the subcontracting agreement is established. Copies of all laboratory *QA Management Plans* for each project laboratory are maintained by the LC. The QATS contractor will also review the laboratory *QA Management Plan* during the annual project laboratory audit (see Section 11.1.2 below).

9.5.2 *Certifications*

All analytical laboratories participating in the analysis of samples for the Libby project are subject to national, local, and project-specific certifications and requirements. Each laboratory is accredited by the National Institute of Standards and Technology (NIST)/NVLAP for the analysis of airborne asbestos by TEM and/or analysis of bulk asbestos by PLM. This includes the analysis of NIST/NVLAP standard reference materials, or other verified quantitative standards, and successful participation in two proficiency rounds per year each of bulk asbestos by PLM and airborne asbestos by TEM supplied by NIST/NVLAP.

Copies of recent proficiency examinations from NVLAP or an equivalent program are maintained by each participating analytical laboratory. Many of the laboratories also maintain certifications from other state and local agencies. Copies of all proficiency examinations and certifications are also maintained by the LC.

Each laboratory working on the Libby project is also required to pass an onsite EPA laboratory audit. The details of this EPA audit are discussed in Section 11.1.2. The LC also reserves the right to conduct any additional investigations deemed necessary to determine the ability of each laboratory to perform the work. Each laboratory also maintains appropriate certifications from the state and possibly other certifying bodies (e.g., New York State Department of Health) for methods and parameters that may also be of interest to the Libby project. These certifications require that each laboratory has all applicable state licenses and employs only qualified personnel. Laboratory personnel working on the Libby project are reviewed for requisite experience and technical competence to perform asbestos analyses. Copies of personnel resumes are maintained for each participating laboratory by the LC in the Libby project file.

9.5.3 *Laboratory Team Training/Mentoring Program*

Initial Mentoring

The orientation program to help new laboratories gain the skills needed to perform reliable analyses at the Site involves successful completion of a training/mentoring program that was developed for new laboratories before their analysis of Libby field samples. All new laboratories are required to participate in this program. The training program includes a rigorous 2-3 day period of onsite training provided by senior personnel from those laboratories already under contract on the Libby project, with oversight by the QATS contractor. The tutorial process includes a review of morphological, optical, chemical, and electron diffraction characteristics of LA, as well as training on project-specific analytical methodology, documentation, and administrative procedures used on the Libby site. The mentor will also

review the analysis of at least one sample by each type of analytical method with the trainee laboratory.

Site-Specific Reference Materials

TEM - Because LA is not a common form of asbestos, USGS prepared site-specific reference materials using LA collected at the Libby mine site (EPA 2008a). Upon entry into the Libby program, each laboratory is provided samples of these LA reference materials. Each laboratory is required to analyze multiple LA structures present in these samples by TEM to become familiar with the physical and chemical appearance of LA and to establish a reference library of LA energy dispersive spectrometry (EDS) spectra. These laboratory-specific and instrument-specific LA reference spectra (EPA 2008b) serve to guide the classification of asbestos structures observed in Libby field samples during TEM analysis.

PLM - USGS has also prepared site-specific reference materials of LA in soil for use during PLM-VE analysis (EPA 2008a). These reference materials were prepared by adding aliquots of LA spiking material to uncontaminated Libby soils to obtain nominal LA concentrations of 0.2% and 1.0% (by weight). Each laboratory was provided with samples of these reference materials for use in training PLM analysts in the visual area estimation of LA levels in soil. In addition, aliquots of these reference materials (as well as other spiked soils) are also utilized as PE standards to evaluate PLM laboratory accuracy.

Regular Technical Discussions

On-going training and communication is an essential component of QA for the Libby project. To ensure that all laboratories are aware of any technical or procedural issues that may arise, a regular teleconference is held between the EPA, their contractors, and each of the participating laboratories. Other experts (e.g., USGS) are invited to participate when needed. These calls cover all aspects of the analytical process, including sample flow, information processing, technical issues, analytical method procedures and development, documentation issues, project-specific laboratory modifications, and pertinent asbestos publications.

Professional/Technical Meetings

Another important aspect of laboratory team training has been the participation in technical conferences. The first of these technical conferences was hosted by USGS in Denver, Colorado, in February 2001, and was followed by another held in December 2002. The Libby laboratory team has also convened on multiple occasions at the ASTM Johnston Conference in Burlington, Vermont, including in July 2002, July 2005, July 2008, and July 2011, and at the Michael E. Beard Asbestos Conference in San Antonio, Texas in January 2010. In addition, members of the Libby laboratory team attended an EPA workshop to develop a method to determine whether LA is present in a sample of vermiculite attic insulation held in February 2004 in Alexandria, Virginia.

These conferences enable the Libby laboratory and technical team members to have an on-going exchange of information regarding all analytical and technical aspects of the project, including the benefits of learning about developments by others.

9.5.4 Analyst Training

TEM

All TEM analysts for the Libby project undergo extensive training to understand TEM theory and the application of standard laboratory procedures and methodologies. The training is typically performed by a combination of personnel, including the laboratory manager, the laboratory QAM, and senior TEM analysts.

In addition to the standard TEM training requirements, trainees involved with the Libby project must familiarize themselves with Site-specific method deviations, project-specific documents, and visual references. Standard samples that are often used during TEM training include known pure (traceable) samples of chrysotile, amosite, crocidolite, tremolite, actinolite and anthophyllite, as well as fibrous non-asbestos minerals such as vermiculite, gypsum, antigorite, kaolinite, and sepiolite. New TEM analysts on the Libby project are also required to perform an *EDS Spectra Characterization Study* (EPA 2008b) on the LA-specific reference materials provided during the initial training program to aide in LA mineralogy recognition and definition. Satisfactory completion of each of these tasks must be approved by a senior TEM analyst.

All TEM analysts are also trained in the Site-specific laboratory QA/QC program requirements for TEM. The entire program is discussed to ensure understanding of requirements and responsibilities. In addition, analysts are trained in the project-specific reporting requirements and data reporting tools utilized in transmitting results. Upon completion of training, the TEM analyst is enrolled as an active participant in the Libby laboratory program.

A training checklist or logbook is used to assure that the analyst has satisfactorily completed each specific training requirement. It is the responsibility of the laboratory QAM to ensure that all TEM analysts have completed the required training requirements.

PLM

All PLM analysts for the Libby project are expected to be familiar with routine chemical laboratory procedures, principles of optical mineralogy, and proficient in EPA Method 600/R-93/116, NIOSH Method 9002, CARB Method 435, and Site-specific SOPs SRC-LIBBY-01 and SRC-LIBBY-03. Analysts with less than one year of experience specific to the Libby project are required to participate in the laboratory mentoring program to obtain additional guidance and instruction. This training is provided by the laboratory managers and/or senior PLM analysts that are familiar with the types of asbestos and analytical challenges encountered at the Site.

Before performing any Site analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision for the LA-specific reference materials.

Satisfactory completion of each of these training tasks must be approved by a senior PLM analyst. A training checklist or logbook is used to ensure that the analyst has satisfactorily completed each specific training requirement. It is the responsibility of the laboratory QAM to ensure that all analysts have completed the required training requirements.

9.5.5 Instrument Calibration

The laboratory manager is responsible for ensuring that all laboratory instruments used for this project are maintained and calibrated in accordance with the manufacturer instructions. If any deficiencies in instrument function are identified, all analyses shall be halted until the deficiency is corrected. The laboratory shall maintain a log that documents all routine maintenance and calibration activities, as well as any significant repair events, including documentation that the deficiency has been corrected.

9.5.6 Inspection of Laboratory Supplies and Consumables

The laboratory manager is responsible for ensuring that all reagents and disposable equipment used in this project are free of asbestos contamination. This is demonstrated by the collection of laboratory blank samples.

9.5.7 Laboratory QC Analyses

TEM

The Libby-specific QC requirements for TEM analyses of asbestos are patterned after the requirements set forth by NVLAP. In brief, there are three types of laboratory-based QC analyses that are performed for TEM – laboratory blanks, recounts, and repreparations. Detailed information on the Libby-specific requirements for each type of TEM QC analysis, including the minimum frequency rates, selection procedures, acceptance criteria, and corrective actions are provided in the most recent version of Libby Laboratory Modification LB-000029, with the following investigation-specific modifications:

- Laboratory QC sample frequency requirements for TEM should be applied on an OU3-specific and medium-specific basis, rather than “across all media” as specified in LB-000029.
- Inter-laboratory analyses should be performed at a minimum frequency of 2% and repreparations at a minimum frequency of 4%.

PLM

Laboratory QC for PLM-Grav is ensured through compliance with laboratory-based QC requirements for the NIOSH Method 9002, as specified by NVLAP. No additional project-specific QC requirements have been established for PLM-Grav.

The Libby-specific QC requirements for PLM-VE are specified in SOP SRC-LIBBY-03. Three types of laboratory-based QC analyses are performed for PLM-VE, including laboratory duplicates, inter-laboratory analyses, and PE standards. Detailed information on the Libby-specific requirements for each type of PLM-VE QC analysis, including the minimum frequency rates, selection procedures, acceptance criteria, and corrective actions are provided in SOP SRC-LIBBY-03, with the following investigation-specific modifications:

- Laboratory QC sample frequency requirements for PLM-VE should be applied on an OU3-specific basis.

With the exception of inter-laboratory analyses, it is the responsibility of the laboratory manager to ensure that the proper number of PLM-VE QC analyses are completed. Inter-laboratory analyses for PLM-VE will be selected *post hoc* by the QATS contractor (or their designate) in accordance with the selection procedures presented in SOP SRC-LIBBY-03. The LC will provide the list of selected inter-laboratory analyses to the laboratory manager and will facilitate the exchange of samples between the analytical laboratories.

9.6 Analytical Laboratory QA/QC Procedures for Non-Asbestos

9.6.1 Laboratory Quality Assurance Management Plan

As noted above, each analytical laboratory has developed a laboratory-specific *QA Management Plan* that provides a detailed description of the procedures and policies that are in place at their laboratory to ensure laboratory quality. This laboratory *QA Management Plan* will include information on standard laboratory methods and SOPs, instrument testing, inspection, maintenance, and calibration requirements, procedures for inspection of supplies and consumables, analyst training, facility contamination monitoring, and internal auditing.

9.6.2 Instrument Calibration

Analytical instruments will be calibrated in accordance with the referenced analytical methods. All target analytes that are reported to the EPA will be present in the initial and continuing calibrations, and these calibrations must meet the acceptance criteria specified in referenced methods. Records of standard preparation and instrument calibration will be maintained by the contract laboratory. Records will unambiguously trace the preparation of standards and their

use in calibration and quantitation of sample results. Calibration standards will be traceable to standard materials.

Analyte concentrations are determined with either calibration curves (linear regression) or response factors (RFs). All correlation coefficients for linear regression calibration curves or relative standard deviation of RFs to determine linearity must meet the acceptability criteria specified within the method. For gas chromatography/mass spectrometry (GC/MS) methods, the average RF from the initial five-point calibration will be used to determine analyte concentrations. The continuing calibration curve will not be used to update the RFs from the initial five-point calibration. GC/MS methods also will meet all instrument performance and/or tuning criteria as specified by the methods.

Calibration procedures for a specific laboratory instrument will consist of initial calibration, initial calibration verification (ICV) and continuing calibration verification (CCV). Calibration protocols included in method references, including calibration frequencies, conditions, and acceptance criteria, will be followed.

Initial Calibration Verification

Initial calibration curves must be verified using a standard made from a source independent of the one used to make the initial calibration standards. All target compounds must be included within the ICV, typically at a concentration around the midpoint of the calibration curve. **Table 9-2** provides control limits and corrective action procedures for “out-of-control” ICV results.

Continuing Calibration and Verification

Initial calibration curves must be verified daily before sample analysis. All target compounds must be included, typically at a concentration around the midpoint of the calibration curve. CCVs are check samples required at frequencies specified in each analytical method, typically at the beginning and end of each analytical sequence and after every ten samples analyzed (as specified in each analytical method). **Table 9-2** provides control limits and corrective action procedures for “out-of-control” CCV results.

9.6.3 Inspection of Laboratory Supplies and Consumables

The laboratory manager is responsible for ensuring that all reagents and disposable equipment used in this project are free of contamination. This is demonstrated by the collection of method blank samples.

9.6.4 Laboratory QC Analyses

The following subsections describe laboratory-based quality control measures used to assess and document the quality of analytical results for non-asbestos analytes. Laboratory QC sample analysis frequencies and control limits used by contracted laboratories will be in accordance with referenced analytical method protocols, and the QC analyses and results will be documented and reported to EPA by the selected laboratory.

Table 9-2 summarizes all laboratory QC measures, control limits, and corrective actions for each non-asbestos analysis method. All laboratory QC data will be reported with results of associated sample analyses to allow for comparison of QC results to the QC criteria specified for this investigation.

Method Blank

Method blanks are designed to measure laboratory-introduced contamination of environmental samples. Method blanks verify that method interferences caused by airborne contaminants, solvents, reagents, glassware, or other sample processing hardware are known and minimized. The blank will be ASTM Type II water (or equivalent) for water samples. The method/reagent blank is processed through all procedures, materials, and laboratory-ware used for sample preparation and analysis.

The frequency for method blank preparation and analysis is a minimum of one per 20 field samples (5%) or per analytical batch, whichever is most frequent. An analytical batch is defined as samples which are analyzed together with the same method sequence and the same lots of reagents and with the manipulations common to each sample within the same time period or in continuous sequential time periods. Samples in each batch are to be of similar composition or matrix.

Table 9-2 provides acceptance criteria and corrective action for “out-of-control” method blanks.

Laboratory Control Samples

Laboratory control samples (LCSs) are designed to check the accuracy of the analytical procedure by measuring a known concentration of an analyte of interest. LCS samples are prepared by spiking clean, laboratory-simulated matrices (reagent-free water or purified solid matrix) with representative analytes at known concentrations that are approximately 10 times greater than the method quantitation limit. These spiked samples are then subjected to the same preparation and analytical procedures as associated environmental samples. An LCS will be analyzed with every analytical batch, and the measured concentrations will be compared to the known, or spiked, concentrations of the LCS to compute a percent recovery value.

LCSs will be analyzed at a minimum frequency of one per every 20 samples or one per analytical batch of no more than 20 samples. **Table 9-2** provides control limits for LCSs. Failure of the LCS to meet recovery criteria requires corrective action before any further analyses can continue.

For some methods, a duplicate of the LCS is also analyzed with each analytical batch and the difference between the LCS and the LCS duplicate (LCSD) indicates the precision of laboratory sample preparation and analysis methods at a known concentration level. **Table 9-2** provides control limits for precision, as measured by the relative percent difference (RPD) of LCS/LCSD results. When LCSD samples are analyzed, the minimum frequency of analysis is one per every 20 samples (5%).

Matrix Spikes/Matrix Spike Duplicates

Matrix spike/matrix spike duplicate (MS/MSD) samples are designed to evaluate the effect of the sample matrix on analytical data, by measuring precision and accuracy from a known concentration of a target analyte that has been added to a particular sample matrix. MS/MSD samples are prepared by spiking environmental field samples with a standard solution containing known concentrations of representative target analytes. The MS/MSD sample pair is prepared from three volumes of an environmental sample. Two portions of the sample (the MS and the MSD) are spiked with the standard solution. The remaining volume is not spiked. The spiked samples are analyzed, and the percent recovery (PR) and RPD between the results of the MS analysis and the MSD analysis are calculated. The unaltered sample volume is analyzed as an ordinary environmental sample.

Typically, additional sample volume will be required to prepare the MS and MSD, especially for analyses of water samples for organic compounds. The COC record will identify for the laboratory which samples are to be used for MS/MSD preparation. Field blanks and field duplicates are not used as MS/MSDs. MS/MSDs will be analyzed at a minimum frequency of one per every 20 samples (5%).

Background and interferences that have an effect on the actual sample analyte will have a similar effect on the spike. The calculated PR of the MS is considered to be a measure of the relative accuracy of the total analytical method (i.e., sample preparation and analysis). The MS is also a measure of the effect of the sample matrix on the ability of the methodology to detect specific analytes. **Table 9-2** provides acceptance criteria and corrective action procedures for “out-of-control” MS/MSD results.

Surrogate Spike Analyses

Surrogate spike analyses are used to determine the efficiency of target analyte recovery during sample preparation and analysis. A surrogate spike is prepared by adding a known amount of

surrogate compound to an environmental sample before extraction. The surrogate compound is selected to exhibit an analytical response that is similar to the response displayed by a target compound during sample analysis. The accuracy of the analytical method is measured using the calculated percent recovery of the spiking compound. Poor reproducibility and percent recovery during surrogate spike analyses may indicate sample matrix effects.

Surrogate compounds are not added to inorganic analyses; however, surrogates are required for most organic analyses. Both environmental and QC samples are spiked with surrogate compounds. Surrogate spike recoveries are acceptable if the results of a surrogate spike fall within the control limits established by laboratory QC protocol. **Table 9-2** provides acceptance criteria and corrective action procedures for “out-of-control” surrogate spike results.

Frequencies for surrogate spike analyses will be consistent with the referenced method protocols.

Internal Standards

Internal standards (ISs) are compounds of known concentrations used to quantify the concentrations of target detections in field and QC samples. ISs are added to all samples after sample extraction or preparation. Because of this, ISs provide for the accurate quantification of target detections by allowing for the effects of sample loss through extraction, purging, and/or matrix effects. ISs are used for any method requiring an IS calibration. Corrective action is required when ISs are out of control. **Table 9-2** provides acceptance criteria and corrective action procedures for “out-of-control” IS results.

9.7 Analytical Turn-Around Time

Analytical turn-around time will be negotiated between the LC and the laboratory, with direction from the EPA RPM. It is anticipated that a turn-around times of 2-3 weeks are acceptable for most samples, but faster turn-around is required for the analysis of the sediment samples collected for the amphibian toxicity tests and the amphibian reference pond selection (e.g., 1-week turn-around). This may be revised as determined necessary by the EPA.

9.8 Custody Procedures

Specific laboratory custody procedures are provided in each laboratory’s *QA Management Plan*, which have been independently reviewed at the time of laboratory procurement. While specific laboratory sample custody procedures may differ between laboratories, the basic laboratory sample custody process is described briefly below.

Upon receipt at the facility, each sample shipment will be inspected to assess the condition of the shipment and the individual samples. This inspection will include verifying sample integrity. The accompanying COC record will be cross-referenced with all of the samples in the shipment. The laboratory sample coordinator will sign the COC record and maintain a copy for their project files.

Depending upon the laboratory-specific tracking procedures, the laboratory sample coordinator may assign a unique laboratory identification number to each sample on the COC. This number, if assigned, will identify the sample through all further handling at the laboratory. It is the responsibility of the laboratory manager to ensure that internal logbooks and records are maintained throughout sample preparation, analysis, and data reporting.

9.9 Record of Modifications

When changes or revisions are needed to improve or document specifics about methods or procedures used by the SPF or analytical laboratory, these changes are documented using a ROM form (see **Appendix E**). The ROM form provides a standardized format for tracking procedural changes in sample preparation and analysis and allows project managers to assess potential impacts on the quality of the data being collected. SPF ROMs will be completed by the appropriate SPF or technical staff. Laboratory ROMs will be completed by the appropriate laboratory or technical staff. Once a form is prepared, it is submitted to the EPA RPM for review and approval. Copies of approved ROMs associated with OU3 samples are available in the OU3 eRoom.

9.10 Results Reporting

Analytical results for asbestos will be recorded and transmitted using the OU3-specific EDDs¹³. Non-asbestos data generated for this project will be transmitted via an EDD spreadsheet. The specific structure and format of this spreadsheet can utilize the standard data reporting templates used by the laboratory (i.e., there are no OU3-specific requirements). Standard project data reporting requirements will be met for this dataset.

Upon completion of the appropriate analyses, EDDs will be posted to the Libby OU3 eRoom within the appropriate turn-around time. Hard copies of all analytical laboratory data packages will be scanned and posted as a portable document format (PDF) to the Libby OU3 eRoom. File names for scanned analytical laboratory data packages will include the laboratory name and the job number to facilitate document organization (e.g., LabX_12345-A.pdf).

¹³ The most current version of all EDDs for OU3 are provided in the OU3 eRoom. (<https://team.cdm.com/eRoom/mt/LibbyOU3>).

9.11 Archival and Final Disposition

All abiotic samples, biota specimens, and TEM grids will be maintained in storage at the Troy SPF or the respective laboratories unless otherwise directed by the EPA. When authorized by the EPA, the SPF or laboratory will be responsible for proper disposal of any remaining samples, sample containers, shipping containers, and packing materials in accordance with sound environmental practice, based on the sample analytical results. The laboratory will maintain proper records of waste disposal methods, and will have disposal company contracts on file for inspection.

10.0 Data Management

All surface water and sediment data generated as part of these ecological studies will be maintained in an OU3-specific Microsoft Access® database in accordance with the OU3-specific data management procedures specified below. All data on fish and amphibian effects will be documented in accordance with the reporting requirements specified in the study-specific protocols (see **Appendix A**). The following sections provide a brief overview of the roles and responsibilities for data management and a summary of the data storage requirements for the OU3 project.

10.1 Roles and Responsibilities

10.1.1 *Field Personnel*

Remedium contractors will perform all sample collection in accordance with this SAP/QAPP. In the field, sample details for surface water and sediment will be documented on hard copy FSDS forms and in field log books. COC information will be documented on hard copy, triplicate forms. FSDS and COC information for surface water and sediment will be manually entered by the field data manager (i.e., Remedium's field contractor) into a field-specific¹⁴ OU3 database using electronic data entry forms. Use of electronic data entry forms ensures the accuracy of data entry and helps maintain data integrity. For example, data entry forms utilize drop-down menus and check boxes whenever possible. These features allow the data entry personnel to select from a set of standard inputs, thereby preventing duplication and transcription errors and limiting the number of available selections (e.g., media types). In addition, entry into a database allows for the incorporation of data entry checks. For example, the database will allow a unique sample ID number to only be entered once, thus ensuring that duplicate records cannot be created.

Entry of FSDS forms and COC information will be completed bi-weekly by the field data manager, or more frequently as conditions permit. Copies of all FSDS forms, COC forms, field log books, and other hard copy field documentation (e.g., study protocol field data sheets) will be scanned and posted as a PDF by the field data manager to the OU3 eRoom on a bi-weekly basis. This eRoom will have controlled access to OU3 project team members (i.e., user name and password are required) to ensure data access is limited to appropriate project-related personnel. File names for scanned FSDS forms, COC forms, field log books, and other field documentation will include the sample date in the format YYYYMMDD to facilitate document organization (e.g., FSDS_20110412.pdf).

¹⁴ The field-specific OU3 database is a simplified version of the master OU3 database. This simplified database includes only the station and sample recording and tracking tables, as well as the FSDS and COC data entry forms.

After FSDS data entry is completed for all collected surface water and sediment samples, a copy of the field-specific OU3 database will be posted by the field data manager to the OU3 eRoom on a weekly basis, or more frequently as conditions permit. The field-specific OU3 database posted to the eRoom site will include the post date in the file name (e.g., FieldOU3DB_20110516.mdb). All FSDS and COC data entry is checked against the field documentation by the OU3 database manager (CDM Smith), or their designate. If errors are identified, the OU3 database manager will request that appropriate changes are made to the field OU3 project database and/or field documentation by the field data manager (Remedium's field contractor) and that revised files be posted to the OU3 eRoom.

Electronic copies of all digital photographs will be posted bi-weekly or more frequently as conditions permit, to the OU3 eRoom. File names for digital photographs should include information on the station identifier and the sample date, as well as other identification information as appropriate (e.g., ST-1_20110412.tif).

Electronic outputs from the temperature logging units will be downloaded and posted to the OU3 eRoom on a bi-weekly basis or more frequently as conditions permit. File names for temperature logging files will include the station identifier and the associated date range (e.g., ST-1_2011_0412-0419.csv).

10.1.2 Troy SPF Personnel

Unless Remedium identifies a suitable sediment sample preparation laboratory that meets the requirements set forth in **Appendix G**, all sediment sample preparation will be performed by the Troy SPF. The Troy SPF utilizes a local SPF Scribe project database to maintain soil sample preparation information. Soil preparation information from the preparation log sheets is entered into the local SPF Scribe project database by SPF personnel. After the data entry is checked against the original forms, it is the responsibility of the SPF manager (or their designate) to publish soil sample preparation information from the local SPF Scribe database to Scribe.NET.

It is the responsibility of the OU3 data manager (CDM Smith) to subscribe to the SPF Scribe project database and upload relevant information on soil sample preparation (e.g., mass associated with each sample fraction) and COC tracking details for OU3 samples into the master OU3 project database.

10.1.3 Laboratory Personnel

Each of the laboratories performing asbestos analyses for this investigation are required to utilize all applicable EDD spreadsheets for data recording and electronic submittals. Upon completion of the appropriate analyses, the EDD spreadsheets, along with scanned copies of all analytical laboratory data packages, will be posted to the OU3 eRoom.

10.1.4 Database Administrator

Day-to-day operations of the master OU3 project database will be under the control of EPA contractors. The primary database administrator (CDM Smith) will be responsible for sample tracking, uploading new data, performing error checks, and making any necessary data corrections. New records will be added to the master OU3 project database within an appropriate time period of data receipt.

10.2 Master OU3 Project Database

The master OU3 project database is a relational Microsoft Access database developed specifically for OU3. The *Libby OU3 Database User's Guide* provides an overview of the master OU3 project database structure and content. The most recent version of this *User's Guide* is provided on the OU3 website.

The master OU3 project database is kept on the CDM Smith server in Denver, Colorado. Incremental backups of the master OU3 project database are performed daily Monday through Friday, and a full backup is performed each Saturday.

10.3 Data Reporting

Field summary reports are prepared by Remedium's field contractor (Golder). Analytical results summaries are included in the OU3 investigation-specific SAP/QAPPs and will be provided in the OU3 Data Summary Report, which are available on the OU3 website. Specialized requests for data summaries may be submitted to the EPA RPM.

10.4 Data Storage

All original data records (both hard copy and electronic) will be cataloged and stored in their original form until otherwise directed by the EPA RPM. At the termination of this project, all original data records will be provided to the EPA RPM for incorporation into the Site project files.

11.0 Assessment and Oversight

Assessments and oversight reports to management are necessary to ensure that procedures are followed as required and that deviations from procedures are documented. These reports also serve to keep management current on field activities.

11.1 Assessments

11.1.1 *Field*

Field surveillances consist of periodic observations performed by the field QAM made to evaluate adherence to investigation-specific governing documents. The schedule for performing field surveillances depends on the duration of the investigation, frequency of execution, and magnitude of process changes. At a minimum, a field surveillance will be performed during the first week of each study. Thereafter, surveillances will be conducted as necessary when field processes are revised or other QA/QC procedures indicates the possibility of deficiencies. When deficiencies are observed during the surveillances, the field QAM will immediately discuss the observation with the field team member and coordinate corrective measures with the FTL, if required. If the observer finds deficiencies across multiple field team members or teams, the FTL will plan and hold a field meeting. At this meeting, the observations made will be discussed and any corrective actions required (e.g., retraining) will be reviewed.

11.1.2 *Troy SPF*

Unless Remedium identifies a suitable sediment sample preparation laboratory that meets the requirements set forth in **Appendix G**, all sediment sample preparation will be performed by the Troy SPF. Internal audits of the SPF are conducted by the SPF QAM periodically to evaluate personnel in their day-to-day activities and to ensure that all processes and procedures are performed in accordance with governing documents and SOPs. All aspects of sample preparation, as well as sample handling, custody, and shipping are evaluated. If any issues are identified, SPF personnel are notified and retrained as appropriate. Audit reports will be completed following each laboratory audit. A copy of the internal audit report, as well as any corrective action reports, will be provided to the LC and the QATS contractor.

Internal audits will be conducted following any significant procedural changes to the soil preparation processes or other SPF governing documents, to ensure the new methods are implemented and followed appropriately.

The Troy SPF is also required to participate in an annual on-site laboratory audit carried out by the EPA through the QATS contract. Audits consist of an evaluation of facility practices and procedures associated with the preparation of soil samples. A checklist of requirements, as derived from the applicable governing documents and SOPs, is prepared by the auditor prior to the audit, and used during the on-site evaluation. Evaluation of the facility is made by

reviewing SPF documentation, observing sample processing, and interviewing personnel.

It is the responsibility of the QATS contractor to prepare an On-site Audit Report following the SPF audit. The On-site Audit Report includes both a summary of the audit results and completed checklist(s), as well as recommendations for corrective actions, as appropriate. Responses from each SPF to any deficiencies noted in the On-site Audit Report are also maintained with the respective reports.

It is the responsibility of the QATS contractor to prepare an On-Site Audit Trend Analysis Report on an annual basis. This report shall include a compilation and trend analysis of the on-site audit findings and recommendations. The purpose of this report is to identify SPF performance problems and isolate the potential causes.

11.1.3 Analytical Laboratory

Each laboratory working on the Libby project is required to participate in an annual onsite laboratory audit carried out by the EPA through the QATS contract. These audits are performed by EPA personnel (and their contractors), that are external to and independent of, the Libby laboratory team members. These audits ensure that each analytical laboratory meets the basic capability and quality standards associated with analytical methods for asbestos used at the Libby site. They also provide information on the availability of sufficient laboratory capacity to meet potential testing needs associated with the Site.

External Audits

Audits consist of several days of technical and evidentiary review of each laboratory. The technical portion of the audit involves an evaluation of laboratory practices and procedures associated with the preparation and analysis of samples for the identification of asbestos. The evidentiary portion of the audit involves an evaluation of data packages, record keeping, SOPs, and the laboratory QA manual. A checklist of method-specific requirements for the commonly used methods for asbestos analysis is prepared by the auditor before the audit, and used during the onsite laboratory evaluation.

Evaluation of the capability for a laboratory to analyze a sample by a specific method is made by observing analysts performing actual sample analyses and interviewing each analyst responsible for the analyses. Observations and responses to questions concerning items on each method-specific checklist are noted. The determination as to whether the laboratory has the capability to analyze a sample by a specific method depends on how well the analysts follow the protocols detailed in the formal method, how well the analysts follow the laboratory-specific method SOPs, and how the analysts respond to method-specific questions.

Evaluation of the laboratory to be sufficient in the evidentiary aspect of the audit is made by reviewing laboratory documentation and interviewing laboratory personnel responsible for maintaining laboratory documentation. This includes personnel responsible for sample check-in, data review, QA procedures, document control, and record archiving. Certain analysts responsible for method quality control, instrument calibration, and document control are also interviewed in this aspect of the audit. Determination as to the capability to be sufficient in this aspect is made based on staff responses to questions and a review of archived data packages and QC documents.

It is the responsibility of the QATS contractor to prepare an On-site Audit Report for each analytical laboratory participating in the Libby program. These reports are handled as business confidential items. The On-site Audit Report includes both a summary of the audit results and completed checklist(s), as well as recommendations for corrective actions, as appropriate. Responses from each laboratory to any deficiencies noted in the On-site Audit Report are also maintained with the respective reports.

It is the responsibility of the QATS contractor to prepare an On-Site Audit Trend Analysis Report on an annual basis. This report shall include a compilation and trend analysis of the onsite audit findings and recommendations. The purpose of this reported is to identify common asbestos laboratory performance problems and isolate the potential causes.

Internal Audits

Each laboratory will also conduct periodic internal audits of their specific operations. Details on these internal audits are provided in the laboratory *QA Management Plan*. The laboratory QAM should immediately contact the LC and the QATS contractor if any issues are identified during internal audits that may impact data quality for OU3 samples.

11.2 Response Actions

Corrective response actions will be implemented on a case-by-case basis to address quality problems. Minor actions taken to immediately correct a quality problem will be documented in the applicable field or laboratory logbooks and a verbal report will be provided to the appropriate manager (e.g., the FTL or LC). Major corrective actions will be approved by the EPA RPM and the appropriate manager prior to implementation of the change. Major response actions are those that address problems that may affect the quality or objective of the investigation, this includes, but is not limited to, quality control issues; missing, broken, or compromised samples; station accessibility issues; and changes in field schedules or analytical deliverable dates. EPA RPM for OU3 will be notified when quality problems arise that cannot be corrected quickly through routine procedures (contact information is provided below):

Christina Progeess
U.S. EPA Region 8
1595 Wynkoop Street
Denver, CO 80202
Tel: (303) 312-6009
Fax: (303) 312-7151
E-mail: progeess.christina@epa.gov

In addition, when modifications to this SAP/QAPP are required, either for field or laboratory activities, a ROM must be completed and approved by the EPA RPM prior to implementation.

11.3 Reports to Management

No regularly scheduled written reports to management are planned as part of this project. However, reports will be provided to management for routine audits and whenever quality problems are encountered. Field and analytical staff will promptly communicate any difficulties or problems in implementation of the SAP/QAPP to the EPA, and may recommend changes as needed. If any revisions to this SAP/QAPP are needed, the EPA RPM will approve these revisions before implementation by field or analytical staff.

12.0 Data Validation and Usability

12.1 Data Review, Verification, and Validation Requirements

12.1.1 Data Review

Data review of project data typically occurs at the time of data reporting by the data users and includes cross-checking that sample numbers and sample dates have been reported correctly and that calculated analytical sensitivities or reported concentration values are as expected. If discrepancies are found, the data user will contact the database administrator (CDM Smith), who will then notify the appropriate entity (field, SPF, or analytical laboratory) to correct the issue.

12.1.2 Criteria for LA Measurement Acceptability

Several factors are considered in determining the acceptability of LA measurements in surface water and fish tissue samples analyzed by TEM. This includes the following:

- *Evenness of filter loading.* This is evaluated using a CHISQ test, as described in ISO 10312 Annex F2. If a filter fails the CHISQ test for evenness, the result may not be representative of the true concentration in the sample, and the results should be given low confidence.
- *Results of QC samples.* This includes both field and laboratory QC samples, such as field and laboratory blank samples, as well as various types of recount and re-preparation analyses. If significant LA contamination is detected in field or laboratory blanks, all samples prepared on that day should be considered to be potentially biased high. If agreement between original analyses and field or laboratory duplicates (i.e., re-preparation or recount analyses) is poor, results for those samples should be given low confidence.

For PLM analyses, the following factors will be considered in determining the acceptability of LA measurements sediment samples:

- *Results of PE standard analyses.* PLM accuracy of visual area estimation results is evaluated using LA-specific PE standards. If the results for these PE standards are not within the project-specific acceptance criteria, results should be given low confidence.
- *Results of QC samples.* This includes field, preparation, and laboratory QC samples. If agreement between original and repeat analyses (i.e., duplicate analyses, inter-

laboratory analyses) is strongly discordant, results for those samples should be given low confidence.

12.1.3 Data Verification Method

Data verification includes checking that results have been transferred correctly from the original hand-written, hard copy field, SPF, and analytical laboratory documentation to the OU3 project database. The goal of data verification is to identify and correct data reporting errors. This process is applicable to any data that are transferred from original hard-copy sheets or forms into an electronic file before data evaluation.

Verification of Asbestos Analytical Data

Because the analytical laboratories utilize the OU3-specific EDD spreadsheets for recording LA analyses, data checking of reported analytical results begins with automatic QC checks that have been built into these spreadsheets. In addition to these automated checks, a detailed manual data verification effort will be performed for 10% of all surface water and sediment samples. This data verification process utilizes Site-specific SOPs developed to ensure TEM and PLM results and field sample information in the OU3 database are accurate and reliable:

- EPA-LIBBY-09 – SOP for TEM Data Review and Data Entry Verification – This Site-specific SOP describes the steps for the verification of TEM analyses, based on a review of the laboratory benchesheets, and verification of the transfer of results from the benchesheets into the project database.
- EPA-LIBBY-10 - SOP for PLM Data Review and Data Entry Verification – This Site-specific SOP describes the steps for the verification of PLM analyses, based on a review of the SPF and laboratory benchesheets, and verification of the transfer of results from the benchesheets into the project database.
- EPA-LIBBY-11 - SOP for FSDS Data Review and Data Entry Verification – This Site-specific SOP describes the steps for the verification of field sample information, based on a review of the FSDS form, and verification of the transfer of results from the FSDS forms into the project database. An FSDS review is performed on all samples selected for TEM or PLM data verification.

The data verification review for LA ensure that any data reporting issues are identified and rectified to limit any impact on overall data quality. If issues are identified during the data verification, the frequency of these checks may be increased as appropriate.

Data verification for LA will be performed by appropriate CDM Smith staff that are familiar with project-specific data reporting, analytical methods, and investigation requirements. The

data verifier will prepare a data verification report (template reports are included in the SOPs) to summarize any issues identified and necessary corrections. A copy of this report will be provided to the appropriate project data manager, LC, and the EPA RPM. It is the responsibility of the OU3 database manager (CDM Smith) to coordinate with the FTL and/or LC to resolve any OU3 project database corrections and address any recommended field, SPF, or laboratory procedural changes from the data verifier. The OU3 database manager is also responsible for electronically tracking in the project database which data have been verified, who performed the verification, and when.

Verification of Other Data

Data verification of other types of data collected as part of this study (e.g., fish, amphibian observations and measurements) will be conducted when data entry from hard copy to electronic format is performed. Following any data entry effort, a second individual should perform a 10% check of entered results to ensure that data are accurate and complete. If any issues are identified, they should be immediately corrected.

12.1.4 Data Validation Method

Unlike data verification, where the goal is to identify and correct data reporting errors, the goal of data validation is to evaluate overall data quality and to assign data qualifiers, as appropriate, to alert data users to any potential data quality issues.

Validation of Asbestos Analytical Data

Data validation will be performed by the QATS contractor (or their designate), with support from technical support staff that are familiar with project-specific data reporting, analytical methods, and investigation requirements. Data validation for asbestos should be performed in basic accordance with the draft *National Functional Guidelines (NFG) for Asbestos Data Review* (EPA 2011), and should include an assessment of the following:

- Internal and external field audit/surveillance reports
- Field ROMs
- Field QC sample results
- Internal and external laboratory audit reports
- Laboratory contamination monitoring results
- SPF and laboratory ROMs
- Preparation and laboratory QC analysis results
- Inter-laboratory analysis results
- PE standard results
- Instrument checks and calibration results

- Data verification results (i.e., in the event that the verification effort identifies a larger data quality issue)

A comprehensive data validation effort of asbestos data for OU3 should be completed quarterly and results should be reported as a technical memorandum. This technical memorandum shall detail the validation procedures performed and provide a narrative on the quality assessment for each type of asbestos analysis, including the data qualifiers assigned, and the reason(s) for these qualifiers. The technical memorandum shall detail any deficiencies and required corrective actions.

Electronic files summarizing the records that have been validated, the date they were validated, any recommended data qualifiers and their associated reason codes should be posted to the OU3 eRoom. It is the responsibility of the OU3 data manager (CDM Smith) to ensure that the appropriate data qualifiers and reason codes recommended by the data validator are added to the project database, and to electronically track in the project database which data have been validated, who performed the validation, and when.

In addition to performing quarterly data validation efforts, it is the responsibility of the QATS contractor (or their designate) to perform a regular evaluation of all blanks, to ensure that any potential contamination issues are quickly identified and resolved. If any blank results are outside the acceptable limits, the QATS contractor should immediately contact the EPA RPM to ensure that appropriate corrective actions are made.

Validation of Non-Asbestos Analytical Data

For non-asbestos analytical data, data validation will be performed in accordance with the most current versions of the EPA NFGs for each analytical method. In brief, the validation process consists of examining the sample data package(s) to determine if the data comply with the requirements specified in the respective NFG. The validator may examine, as appropriate, the reported results, QC summaries, case narratives, COC information, raw data, initial and continuing instrument calibration, and other reported information to evaluate the accuracy and completeness of the data package. During this process, the validator will determine if analytical methodologies were followed and QC requirements were met. The validator may recalculate selected analytical results to verify the accuracy of the reported information, as appropriate, and will assign qualifiers to the data as needed. CDM Smith data validation staff will be responsible for conducting a data validation for 10% of all non-asbestos analytical results from this project.

Electronic files summarizing the records that have been validated, the date they were validated, any recommended data qualifiers and their associated reason codes should be posted to the OU3 eRoom. It is the responsibility of the OU3 data manager (CDM Smith) to ensure that the appropriate data qualifiers and reason codes recommended by the data validator are added to

the project database, and to electronically track in the project database which data have been validated, who performed the validation, and when.

12.2 Reconciliation with Data Quality Objectives

Once all samples have been collected and analytical data has been generated, data will be evaluated to determine if study objectives were achieved. It is the responsibility of data users to perform a data usability assessment to ensure that DQOs have been met, and reported investigation results are adequate and appropriate for their intended use. This data usability assessment should utilize results of the data verification and data validation efforts to provide information on overall data quality specific to each investigation.

The data usability assessment should evaluate results with regard to several data usability indicators, including precision, accuracy and bias, representativeness, comparability, completeness, and whether specified analytic requirements (e.g., sensitivity) were achieved. **Table 12-1** provides detailed information for how each of these indicators may be evaluated for the reported asbestos data. Other evaluation methods may also be appropriate to determine the data usability for non-asbestos data and organism effects endpoints recorded during the ecological investigations. The data usability assessment results and conclusions should be included in any investigation-specific data summary reports.

Non-attainment of project requirements may result in additional sample collection or field observations to achieve project needs.

13.0 References

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Figures

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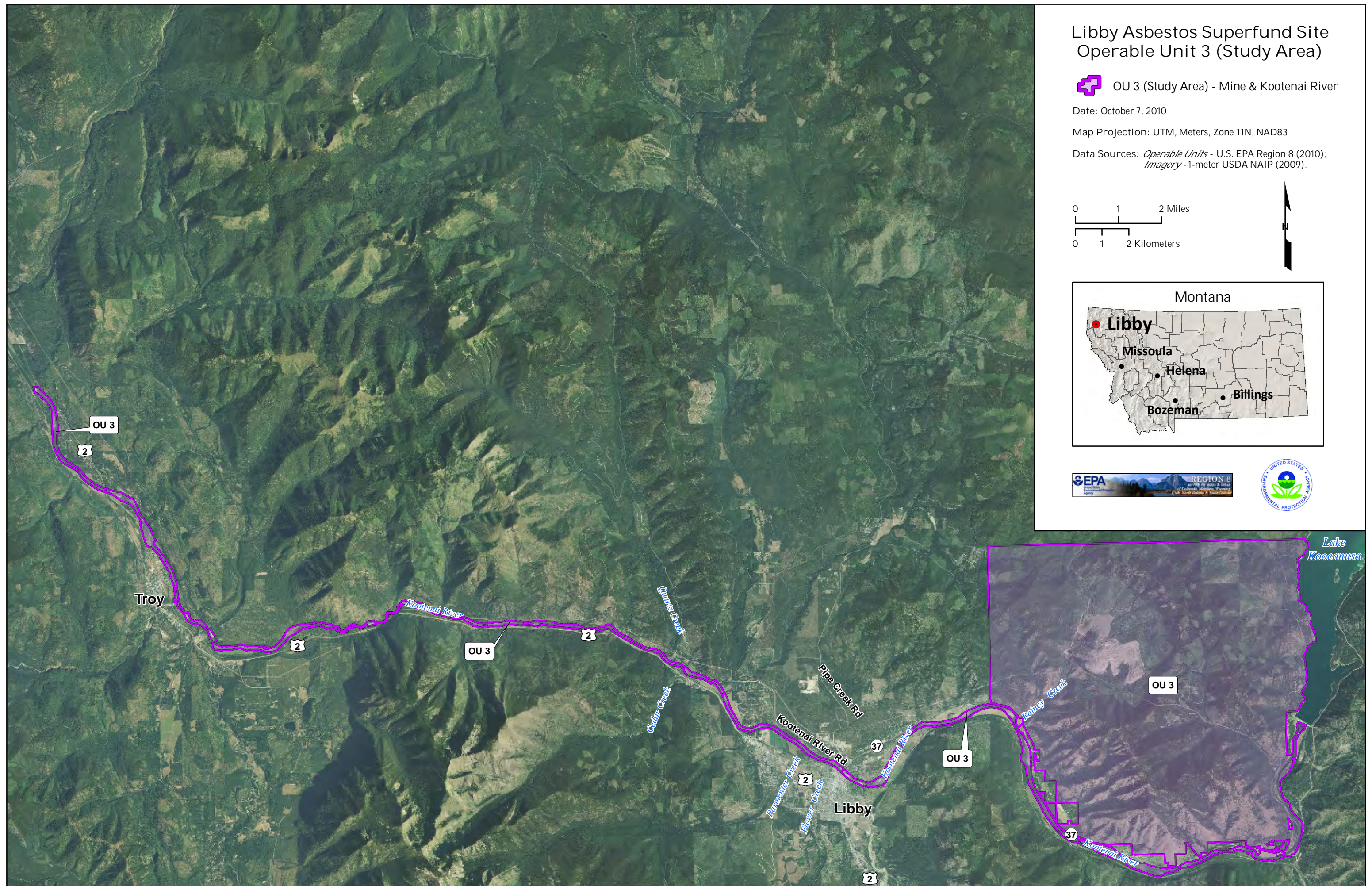
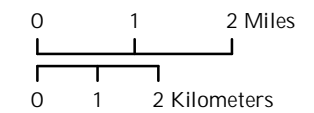
Libby Asbestos Superfund Site Operable Unit 3 (Study Area)

 OU 3 (Study Area) - Mine & Kootenai River

Date: October 7, 2010

Map Projection: UTM, Meters, Zone 11N, NAD83

Data Sources: *Operable Units* - U.S. EPA Region 8 (2010);
Imagery - 1-meter USDA NAIP (2009).



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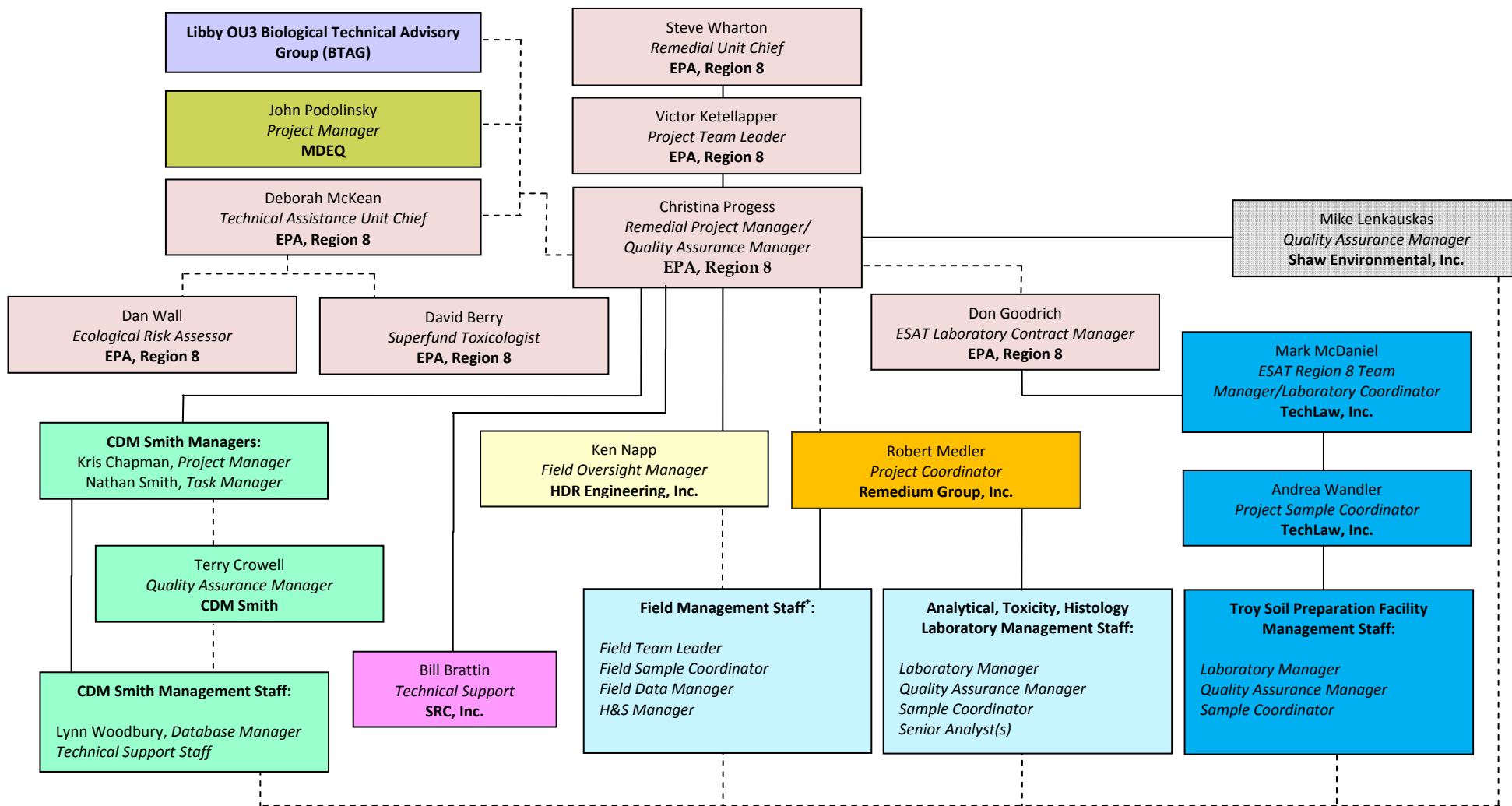
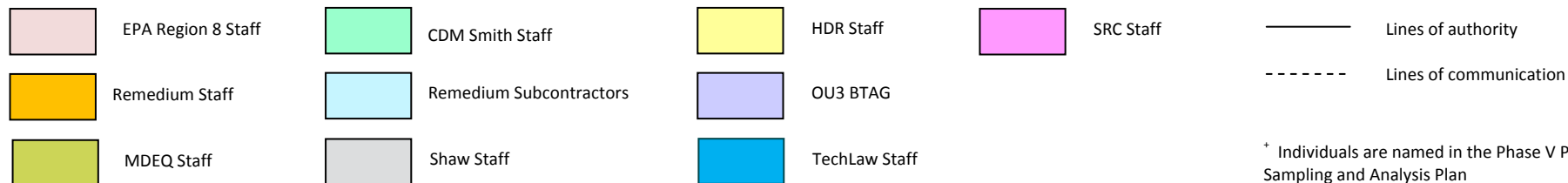
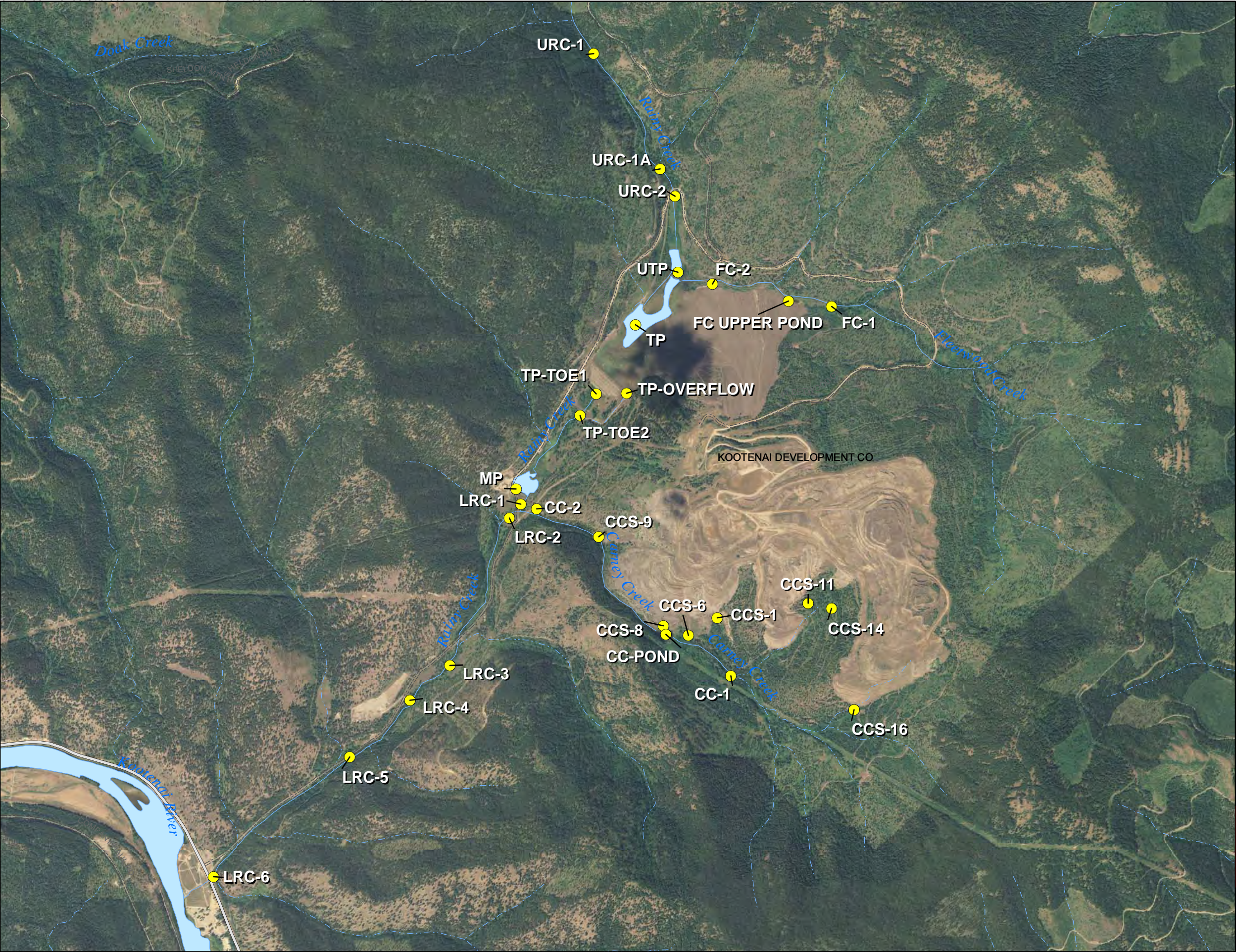


FIGURE 2-1. OU3 ORGANIZATIONAL CHART FOR PHASE V PART B



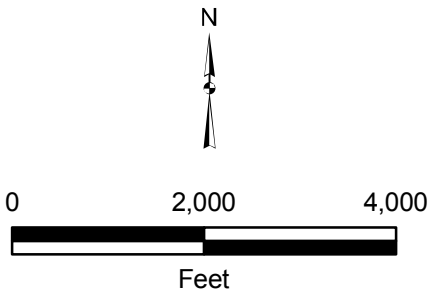
* Individuals are named in the Phase V Part B Sampling and Analysis Plan

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Legend

- Surface Water/Sediment Sampling Location
- ==== County Road
- ==== Primary Road
- Open Water
- Perennial Stream
- - - Intermittent Stream



LIBBY MONTANA SUPERFUND SITE
OPERABLE UNIT 3

FIGURE 3-1
AQUATIC SAMPLING LOCATIONS IN
THE RAINY CREEK WATERSHED

PROJECT: 0100-008-900 JAN. 29, 2008		
REV: 0	BY: VFS	CHECKED: ACK



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FIGURE 3-2 GOSNER STAGES OF AMPHIBIAN DEVELOPMENT

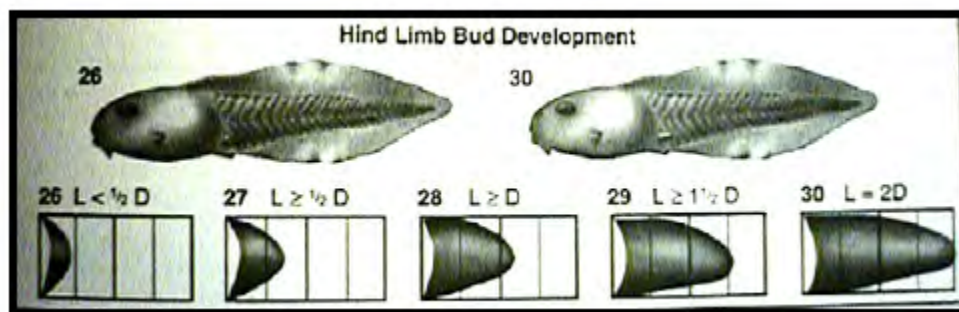
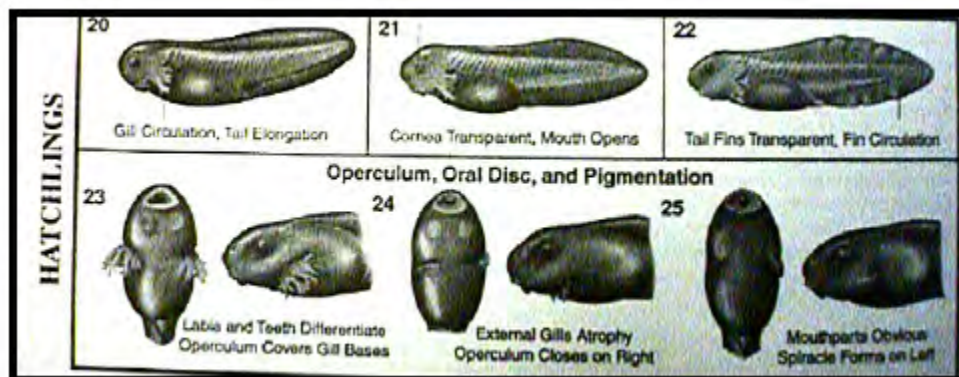
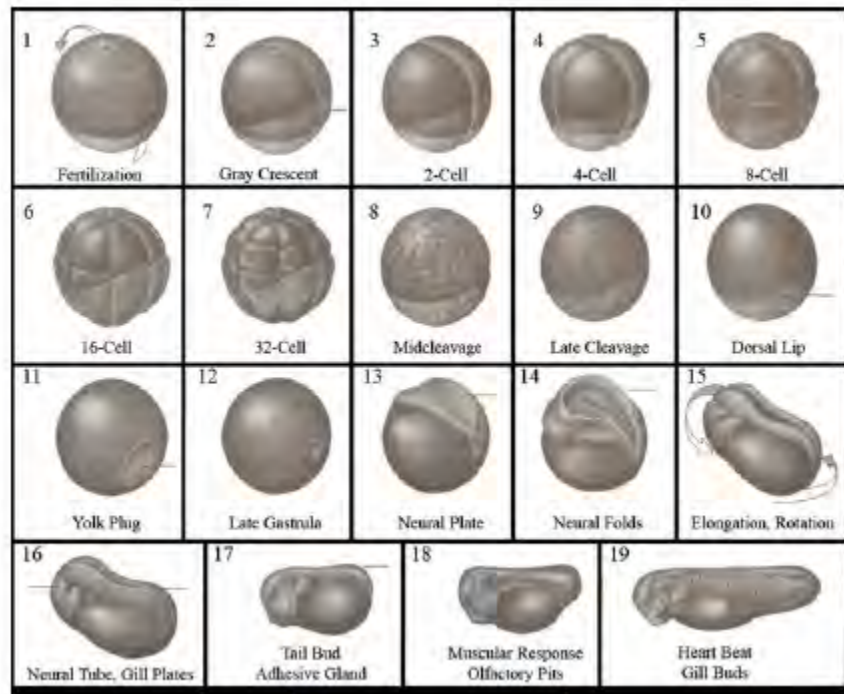
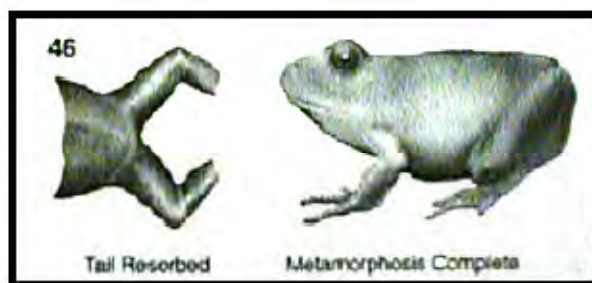
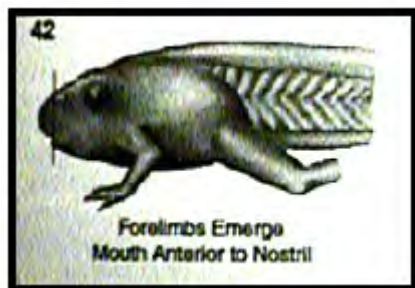
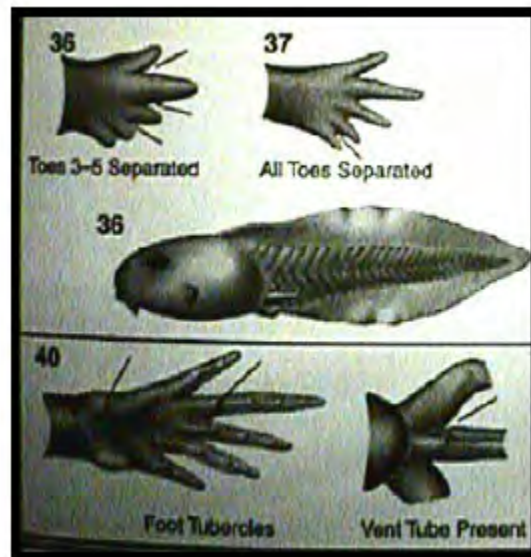
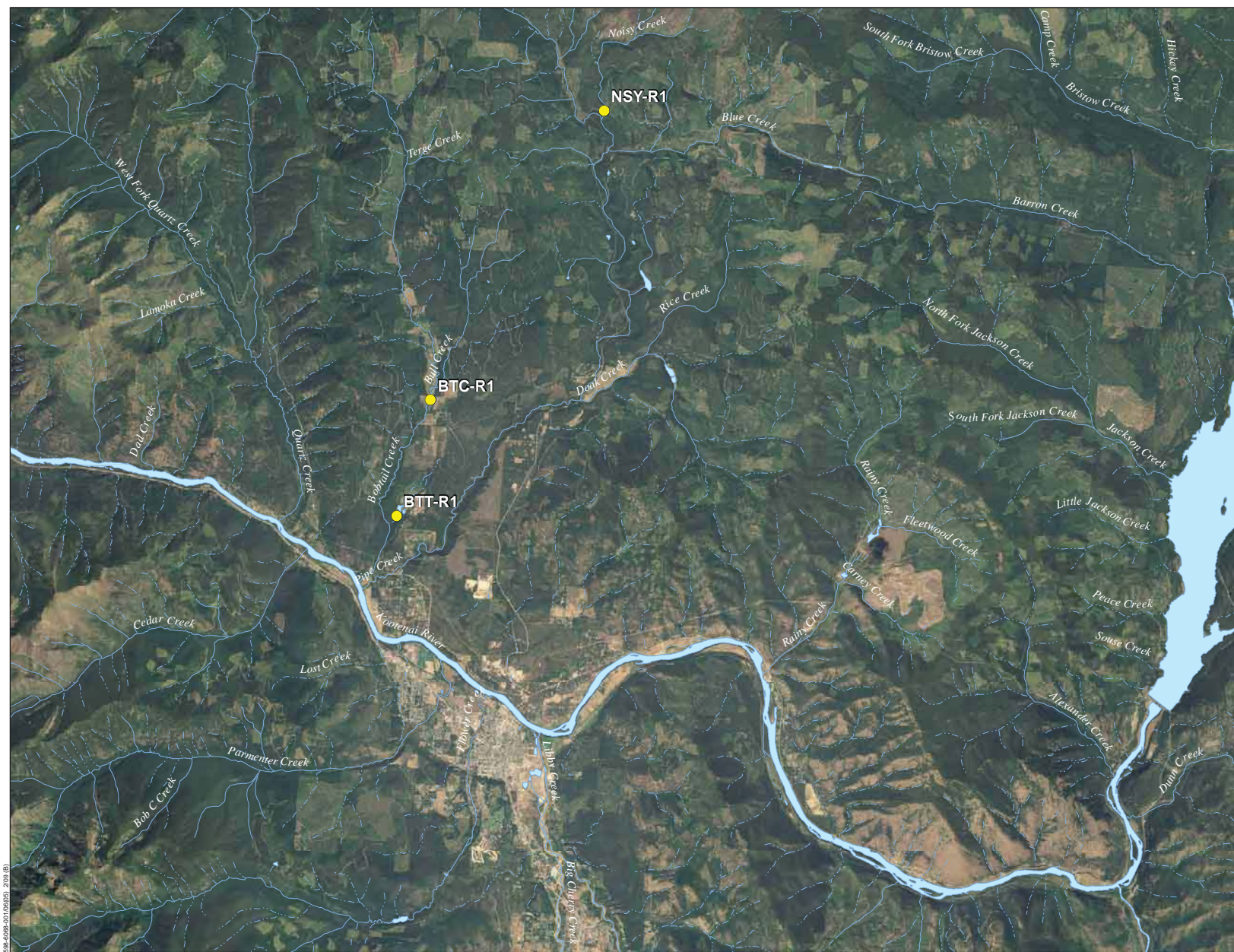


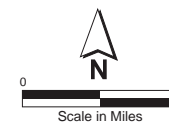
FIGURE 3-2 GOSNER STAGES OF AMPHIBIAN DEVELOPMENT (cont.)



Source: Gosner (1960)



Parametrix



Legend

● Aquatic Reference Location

Note: Samples were collected from reference locations NSY-R1 and BTT-R1 only.

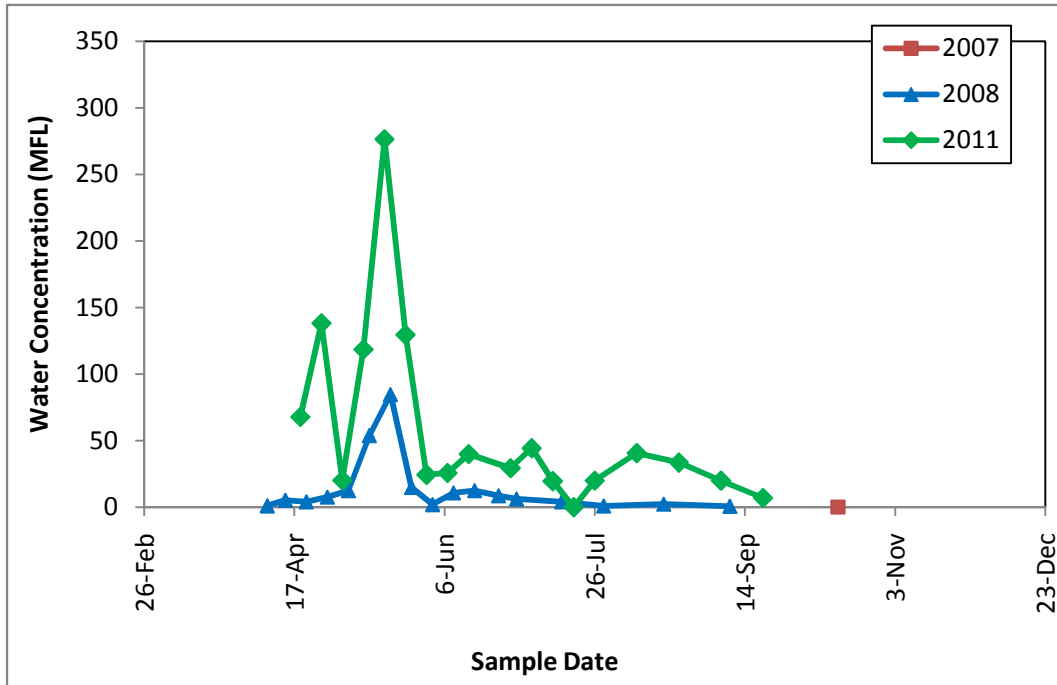
Source: EPA, 2008

Figure 4-1
Libby Montana Superfund Site
Operable Unit 3,
Aquatic Reference Locations

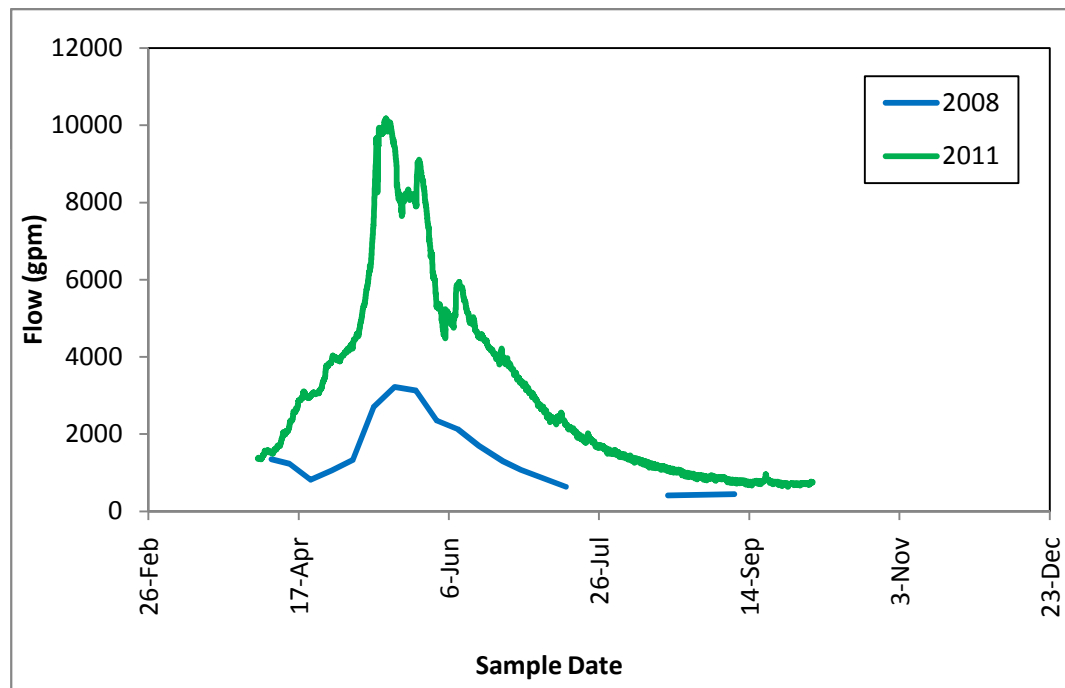
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FIGURE 5-1. MEASURED LA CONCENTRATIONS AND FLOW AT LRC-6

Panel A: Total LA Water Concentration



Panel B: Flow

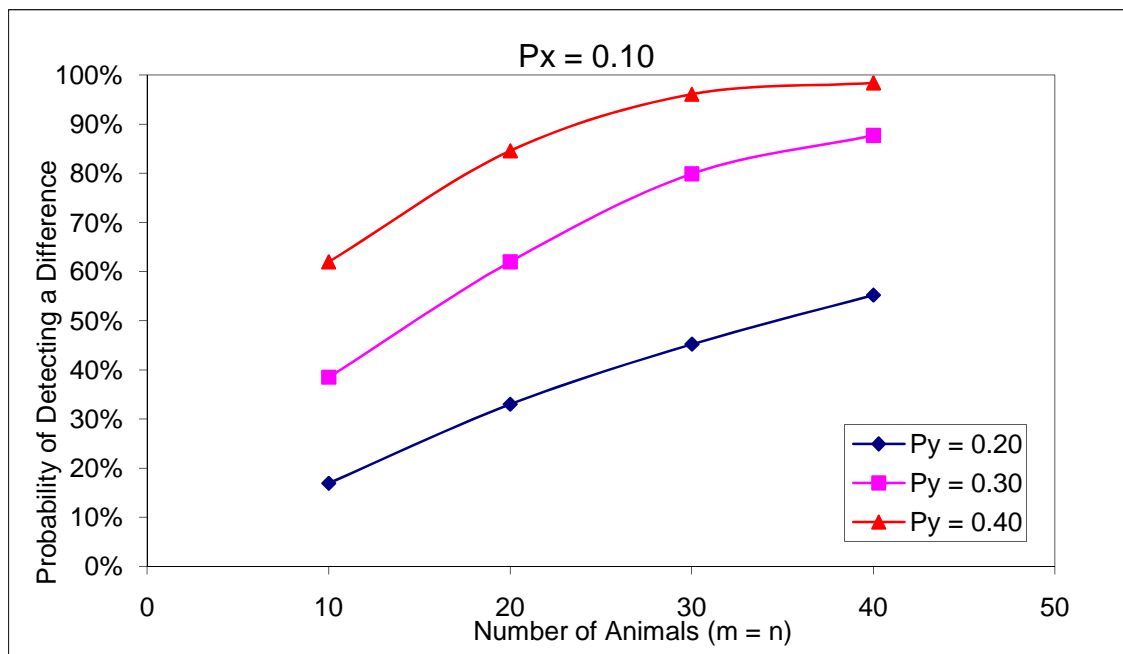
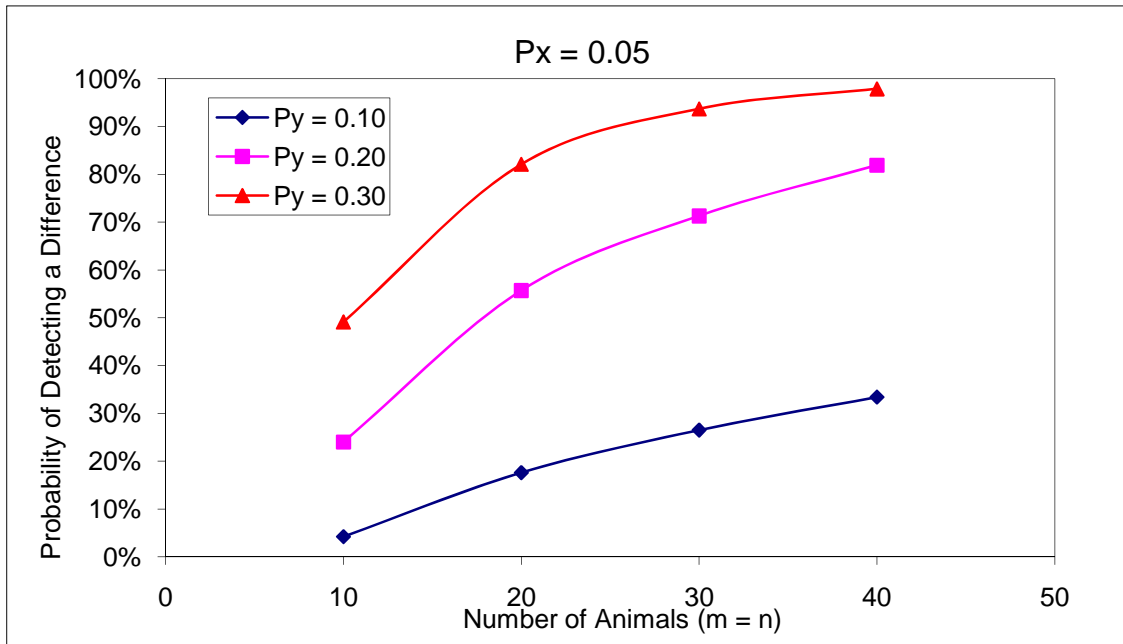


GPM = gallons per minute

MFL = million fibers per liter

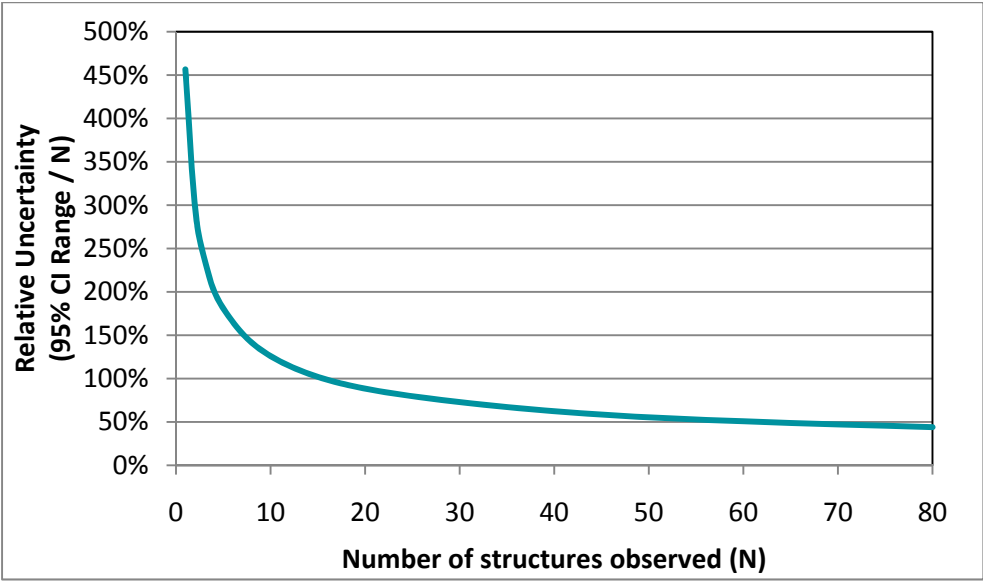
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Figure 6-1
Probability of Observing a Statistically Significant Difference as a Function of Sample Size



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**FIGURE 9-1. RELATIONSHIP BETWEEN NUMBER OF STRUCTURES OBSERVED
AND RELATIVE UNCERTAINTY**



CI = confidence interval

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Tables

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Table 1-1. QA/R5 QAPP Element Cross-Reference

QA/R-5 QAPP Element	Phase V Part B SAP/QAPP Document Location
Group A. Project Management	
A1. Title & Approval Sheet	Approval page (pg. 3)
A2. Table of Contents	Table of Contents (pg. 8-11)
A3. Distribution List	Distribution List (pg. 5-6)
A4. Project/Task Organization	Section 2
A5. Problem Definition & Background	Sections 1.1-1.4; Sections 3.1, 4.1, 5.1, 6.1, and 7.1
A6. Project/Task Description	Sections 3.1.4, 4.1.4, 5.1.4, 6.1.4, and 7.1.4
A7. Quality Objectives & Criteria	Sections 3.1, 4.1, 5.1, 6.1, 7.1, and 9.1.1, Table 12-1
A8. Special Training/Certifications	<u>Field</u> : Section 8.1 <u>Troy SPF</u> : Section 9.4.1 <u>Lab</u> : Sections 9.5.2 to 9.5.4
A9. Documentation & Records	<u>Field</u> : Section 8.5.2 <u>Lab</u> : Section 9.10 Section 10
Group B. Data Generation & Acquisition	
B1. Sampling Process Design (Experimental Design)	Sections 3 to 7
B2. Sampling Methods	Sections 3 to 7
B3. Sample Handling & Custody	Sections 8.6.1 to 8.6.7
B4. Analytical Methods	Sections 9.1 to 9.3, 9.7, and 9.11
B5. Quality Control	<u>Field</u> : Sections 3.4, 4.4, 5.4, 6.4, and 7.4 <u>Troy SPF</u> : Section 9.4 <u>Lab</u> : Sections 9.5 to 9.6
B6. Instrument/Equipment Testing, Inspection, & Maintenance	<u>Field</u> : Section 8.4 <u>Lab</u> : Sections 9.5.5 and 9.6.2
B7. Instrument/Equipment Calibration & Frequency	<u>Field</u> : Section 8.4 <u>Lab</u> : Sections 9.5.5 and 9.6.2
B8. Inspection/Acceptance of Supplies & Consumables	<u>Field</u> : Section 8.3 <u>Lab</u> : Sections 9.5.6 and 9.6.3
B9. Non-direct Measurements	NA
B10. Data Management	Sections 10.1 to 10.4
Group C. Assessment & Oversight	
C1. Assessments & Response Actions	Section 11
C2. Reports to Management	Section 11.3
Group D. Data Validation & Usability	
D1. Data Review, Verification, & Validation	Section 12.1
D2. Verification & Validation Methods	Sections 12.1.3 to 12.1.4
D3. Reconciliation with User Requirements	Section 12.2

QAPP – quality assurance project plan

SAP – sampling and analysis plan

SPF – sample preparation facility

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**Table 8-1. Sample Containers, Preservation and Handling Requirements,
and Holding Times for Sediment Samples**

Container Description	Analyses	Analysis Method	Preservation and Handling	Extraction/Analysis Holding Times
Four 4-oz glass jars	Metals/Metalloids	EPA 6010/6020B	Cool 4°C	180 days
	Mercury	EPA 7471A	Cool 4°C	28 days
	Organochlorine Pesticides	EPA 8081A	Cool 4°C	14 days/40 days
	Chlorinated Herbicides	EPA 8151A	Cool 4°C	14 days/40 days
	Polychlorinated Biphenyls (PCBs)	EPA 8082	Cool 4°C	14 days/40 days
	Diesel/Gasoline Range Organics	EPA 8015B	Cool 4°C	14 days/40 days
	Semi-volatile Organic Compounds (SVOCs) (a)	EPA 8270C	Cool 4°C	14 days/40 days
	Acid volatile sulfide (AVS)	AVS/TTC	Cool 4°C	14 days
	Ammonia	ASAM 33-7	Cool 4°C	None
	Total Organic Carbon (TOC)	ASAM 29-3	Cool 4°C protect from sunlight and atmospheric oxygen	28 days
	pH	ASAM 10-3	Cool 4°C	None
	Moisture	ASTM D2974	Cool 4°C	180 days
One 500-mL HDPE wide-mouth container	Asbestos	<u>PLM-Grav</u> ; SRC-LIBBY-01 (Rev. 3) <u>PLM-VE</u> ; SRC-LIBBY-03 (Rev. 3)	None	None

(a) CLP analyte list

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Table 9-1
Overview of Phase V Part B Samples Collected for Analysis

Study	Sampling Locations	Sampling Overview	N field samples	Analysis Method
Amphibian Laboratory Toxicity	Site: TP-TOE2, CC-1	Pre-test Sediment: 3 "lots", 5 replicates/lot per station	30	PLM-VE
		1 composite per station (across 3 lots)	2	Non-asb.*
		Overlying water (@ start/end of study for each replicate)	32	Water qual.**
		Chamber sediment (@ end of study for site replicates)	4	PLM-VE
Amphibian Field Study	On-Site: Tailings Impoundment, Mill Pond, Fleetwood Pond, Carney Pond	Pre-selection Sediment: 1 per station	4	PLM-VE
			4	Non-asb.*
		Surface Water: 1 per week per station (May-Aug)	64	TEM (ISO)
		Sediment: 2 per station (1 @ start & end of study)	8	PLM-VE
	Candidate Ref: 6 locations	Pre-selection Sediment: 1 per station	6	PLM-VE
			6	Non-asb.*
Caged Fish Study (eggs)	Site: 3 LRC segments	Surface Water: 2 per station (1 @ start & end of study)	6	TEM (ISO)
		Sediment: 2 per station (1 @ start & end of study)	6	PLM-VE
	Ref: NSY, URC	Pore Water (inside box): 2 per segment per week (8 weeks)	48	TEM (ISO)
		Pore Water (outside box): 2 per segment (2 sampling events)	6	TEM (ISO)
		Surface Water (outside box): 2 per segment (2 sampling events)	6	TEM (ISO)
Caged Fish Study (fry)	Ref: NSY, URC	Pore Water (inside box): 1 per station per week	16	TEM (ISO)
	Site: 3 LRC segments	Surface Water (inside box): 2 per segment per week (4 weeks)	24	TEM (ISO)
	Ref: NSY, URC	Surface Water (inside box): 1 per station per week	8	TEM (ISO)
Fish Lesion Study	Site: TPTOE-2, LRC-1, LRC-2, LRC-3, LRC-5	Fish: 4 per station (goal)	20	no analysis to be performed
	Ref: NSY, URC	Fish: 20 per station	40	

*See Section 9.3.2 for the list of analytes

**See Section 9.3.1 for the list of analytes

 = Rapid turn-around analysis time is needed

Revision 1 (May 22, 2012)

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Table 9-2. Non-Asbestos Laboratory Quality Control Measures by Analytical Method

Analytical Method^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
ICP Metals SW-846 6010	Initial calibration (1 point + blank minimum)	Daily prior to analysis	Correlation coefficient (r) ≥ 0.995	<ul style="list-style-type: none"> Recalibrate
	Interference check standard (ICS)	Beginning and end of each analytical run	Results +/- 20% of true value	<ul style="list-style-type: none"> Terminate analysis Recalibrate instrument Reanalyze all samples back to last acceptable ICS
	Initial calibration verification (ICV)	After calibration, prior to sample analysis	Results <10% from calibration standard	<ul style="list-style-type: none"> Reanalyze ICV Recalibrate, if ICV still out
	Continuing calibration verification (CCV)	Every 10 samples and end of analytical sequence	Results < 10% from calibration standard	<ul style="list-style-type: none"> Reanalyze affected samples back to the last acceptable CCV
	Calibration blank - Initial calibration blank (ICB), Continuing calibration blank (CCB)	After initial calibration verification, each subsequent calibration verification, and at the end of the run	<3x the Method detection limit (MDL)	<ul style="list-style-type: none"> Reanalyze blank Clean system Reanalyze all samples back to last acceptable blank
	Method blank	1 per preparation batch (≤ 20 samples)	< $\frac{1}{2}$ x Practical quantitation limit (PQL)	<ul style="list-style-type: none"> Reanalyze method blank. If fails, analyze a calibration blank Reprep/reanalyze analytical batch as appropriate
	Matrix spike (MS)	1 per preparation batch (≤ 20 samples)	% Recovery +/-25% of actual value	<ul style="list-style-type: none"> Assess data (4 x rule) If LCS recoveries are within acceptance criteria, then matrix interference may be suspected Reanalyze reprep once if matrix is not a factor Narrate all outliers
	Matrix spike duplicate (MSD)	1 per preparation batch (≤ 20 samples)	RPD <20%	<ul style="list-style-type: none"> Same as MS
	Laboratory Control Sample (LCS)	1 per preparation batch (≤ 20 samples)	% Recovery +/- 20% of actual value	<ul style="list-style-type: none"> Reanalyze LCS Reprep/reanalyze LCS and affected samples Narrate all outliers
ICP-MS Metals SW-846 6020	Mass calibration and resolution check (4 replicates)	Daily prior to analysis	Mass calibration < 0.1 amu; resolution <0.9 amu at 10% peak height; RSD <5%	<ul style="list-style-type: none"> Recalibrate
	Initial multipoint calibration (1 point + blank minimum); average of 3 integrations	Daily prior to analysis	None	<ul style="list-style-type: none"> None
	Initial calibration verification (ICV); mid-level standard second source	After calibration, prior to sample analysis	$\pm 10\%$ from true value	<ul style="list-style-type: none"> Reanalyze ICV Recalibrate, if ICV still out
	Continuing calibration verification (CCV)	Every 10 samples and end of run sequence	$\pm 10\%$ from true value	<ul style="list-style-type: none"> Reanalyze affected samples back to the last acceptable CCV

Analytical Method ^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
ICP-MS Metals SW-846 6020	Interference check solution	At beginning of analytical sequence or once every 12 hours, whichever is more frequent	Recoveries +/- 20% of theoretical value	<ul style="list-style-type: none"> Internal QC review only; flag data to indicate interference
	Internal Standards	Every CCV, ICB/CCB	Recoveries +/- 20% of initial calibration	<ul style="list-style-type: none"> Recalibrate and verify calibration Reanalyze affected samples
		Every sample	Recoveries 30-120% for samples	<ul style="list-style-type: none"> Dilute sample 5x and reanalyze Repeat until within limits
	Calibration blank Initial calibration blank (ICB) Continuing calibration blank (CCB)	After initial calibration and each subsequent calibration verification	< 3 x Method detection limit (MDL)	<ul style="list-style-type: none"> Reanalyze blank Clean system if still out Reanalyze affected samples back to the last acceptable CCB
	Method blank	1 per preparation batch (≤ 20 samples)	< ½ x PQL	<ul style="list-style-type: none"> Reanalyze method blank. If fails, analyze a calibration blank Reprep/reanalyze analytical batch as appropriate
	Matrix spike (MS)	1 per preparation batch (≤ 20 samples)	% Recovery +/- 25% of true value	<ul style="list-style-type: none"> Assess data Reanalyze MS if matrix is not a factor
	Matrix spike duplicate (MSD) or Matrix duplicate (MD)	1 per preparation batch (≤ 20 samples)	RPD < 20% (for values > 100 x MDL)	<ul style="list-style-type: none"> Same as MS
	Post-digestion spike addition	As necessary to assess matrix interference	% Recovery +/- 25% of actual value	<ul style="list-style-type: none"> Perform dilution test Or, perform method of standard addition
	Dilution test	1 per 20 samples	% Recovery +/- 10% of true value	<ul style="list-style-type: none"> Use method of standards addition
	Laboratory control sample (LCS)	1 per preparation batch (≤ 0 samples)	% Recovery within +/- 20% of true value	<ul style="list-style-type: none"> Reanalyze LCS Reprep/reanalyze LCS and affected samples Narrate all outliers
Mercury SW-846 7470A/7471A	Initial multipoint calibration (3 point + blank minimum)	Daily, prior to analysis	Correlation coefficient (r) ≤ 0.995	<ul style="list-style-type: none"> Recalibrate
	Initial calibration verification (ICV); mid-level standard	After calibration, prior to sample analysis	± 20% of true value	<ul style="list-style-type: none"> Reanalyze ICV Rerun initial calibration
	Continuing calibration verification (CCV); mid-level standard	Every 10 samples and at end of analytical sequence	± 20% of true value	<ul style="list-style-type: none"> Reanalyze affected samples back to last acceptable CCV
	Calibration blank (ICB/CCB)	After calibration, and after each subsequent calibration verification	< ½ x PQL	<ul style="list-style-type: none"> Reanalyze blank Clean system if still out Reanalyze affected samples back to last acceptable CCB
	Method blank	1 per preparation batch (≤ 20 samples)	< ½ x PQL	<ul style="list-style-type: none"> Reanalyze method blank. If fails, analyze a calibration blank Reprep/reanalyze analytical batch as appropriate

Analytical Method ^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
Mercury SW-846 7470A/7471A	Matrix spike (MS)	1 per preparation batch (≤20 samples)	% Recovery +/- 25% of true value	<ul style="list-style-type: none"> • If LCS recoveries are within acceptance criteria, matrix interference may be suspected • Reprep/reanalyze once if problem cannot be attributed to matrix • Narrate all outliers
	Matrix spike duplicate (MSD)	1 per preparation batch (≤20 samples)	RPD < 20%	<ul style="list-style-type: none"> • Same as MS
	Laboratory control samples (LCS)	1 per preparation batch (≤20 samples)	%Recovery within +/- 20% of true value	<ul style="list-style-type: none"> • Reanalyze LCS • Reprep/reanalyze LCS and affected samples • Narrate all outliers
SW-846 8270C Semi-Volatiles by GC/MS	Tune the instrument using a decafluorotriphenylphosine (DFTPP) standard	Every 12 hours	Must meet the ion abundance criteria specified in the Degradation of DDT ≤ 20% Benzidine and PCP present at normal response without excessive tailing	<ul style="list-style-type: none"> • Retune instrument • Repeat standard analysis • Perform injection port, column maintenance as necessary
	Initial calibration (5 point minimum); includes Calibration Check Compounds (CCC), System Performance Calibration Check (SPCC), and Internal Standard Compounds (IS)	Prior to analysis and as required	% RSD for CCC ≤30%; average RF ≥0.05 for SPCC If % RSD ≤15 % average RF may be used; linear calibration required	<ul style="list-style-type: none"> • Evaluate the system • Repeat calibration
	Continuing calibration verification (CCV); includes CCC, SPCC, and IS	Every 12 hours	CCV percent difference for CCC ≤30%; RF ≥0.05 for SPCC EICP area of each internal standard - 50% to +100% of all IS areas in most recent CCV. Retention time for each internal standard must be within 30 seconds of most recent CCV	<ul style="list-style-type: none"> • Evaluate system/standard • Reanalyze calibration check standard • Repeat the initial calibration as necessary
	Method blank	1 per preparation batch (≤20 samples)	< ½ x PQL	<ul style="list-style-type: none"> • Reanalyze blank • Reprep/reanalyze blank and all associated samples
SW-846 8270C Semi-Volatiles by GC/MS	Internal Standard	Every sample, method blank, LCS and MS/MSD	The EICP area for all internal standards must be within -50% and +100% of most recent CCV Retention time for each internal standard must be within 30 seconds of most recent CCV	<ul style="list-style-type: none"> • Evaluate system/standard • Reanalyze the sample • If still out, report both sets of data

Analytical Method ^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
SW-846 8270C Semi-Volatiles by GC/MS	Surrogate spike	Every sample, method blank, LCS and MS/MSD	No more than one surrogate per fraction outside of acceptance criteria (Refer to Table B1-a) No surrogate below 10% recovery	<ul style="list-style-type: none"> Reanalyze sample once Re-extract and reanalyze if >1 surrogate per fraction outside acceptance limits Narrate all outliers
	Matrix spike (MS)	1 per preparation batch (≤20 samples)	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> Assess data (4x rule) Reanalyze once; if matrix is not a factor If LCS and surrogate recoveries are within acceptance criteria matrix interference maybe suspected Narrate all outliers
	Matrix spike duplicate (MSD) or Matrix Duplicate (MD)	1 per preparation batch (≤20 samples)	% Recovery and/or RPD within QC acceptance criteria	<ul style="list-style-type: none"> Same as MS
	Laboratory control sample	1 per preparation batch (≤20 samples)	% Recovery within project QC acceptance criteria for all spiked analytes	<ul style="list-style-type: none"> Reanalyze LCS Re-prep/reanalyze LCS and all associated samples Narrate all outliers
SW-846 8082 Polychlorinated biphenyls (PCBs) by Gas Chromatography	Initial calibration (5 point minimum) Lowest standard at or below PQL; Expected Aroclors or Aroclor 1016/1260 five-point if unknown with single-point mid-level standards for other Aroclors for pattern recognition and retention times, or	Prior to analysis and as required	RSD <20%, average calibration factor or response factor(a) may be used; linear calibration required	<ul style="list-style-type: none"> Evaluate the system Repeat initial calibration
	Initial calibration verification (ICV) Mid level standard Expected Aroclors or Aroclor 1016/1260 if unknown	Prior to each 12 hour shift	% Difference ≤15% of expected concentration compared to response from ICAL	<ul style="list-style-type: none"> Evaluate system/standard Reanalyze ICV standard Repeat initial calibration
	Continuing calibration verification (CCV) Mid level standard Expected Aroclors or Aroclor 1016/1260 if unknown	After every 20 samples and at the end of the analytical sequence	% Difference ≤15% of expected concentration compared to response from ICAL for each bracketing standard	<ul style="list-style-type: none"> Evaluate system/standard Reanalyze CCV and samples back to last acceptable CCV
	Retention time windows	Established with each new column installation Updated with each daily initial calibration standard	Retention times must be within retention time window established by the daily initial calibration standard Every CCV and every sample	<ul style="list-style-type: none"> Evaluate system/standard; pattern recognition may be sufficient Reanalyze CCV/affected samples
SW-846 8082 Polychlorinated biphenyls (PCBs) by Gas Chromatography	Method Blank	1 per preparation batch (≤20 samples)	< ½ x PQL	<ul style="list-style-type: none"> Reanalyze blank Re-prep/reanalyze blank and associated samples
	Surrogate spike DCB (for Aroclors) TCMX (for PCB congeners)	Every sample, method blank, LCS and MS/MSD	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> Re-extract/reanalyze once If still out, report both sets of data Narrate all outliers

Table 9-2 Non-Asb Lab QC.doc

Analytical Method ^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
SW-846 8082 Polychlorinated biphenyls (PCBs) by Gas Chromatography	Matrix spike (MS)	1 per preparation batch (≤20 samples)	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> Assess data (4x rule) If LCS and surrogate recoveries are within acceptance criteria matrix interference maybe suspected Re-extract/reanalyze if matrix is not a factor Narrate all outliers
	Matrix spike duplicate(MSD) or Matrix duplicate (MD)	1 per preparation batch (≤20 samples)	% Recovery and/or RPD within QC acceptance criteria	<ul style="list-style-type: none"> Same as MS
	Laboratory control sample(LCS)	1 per preparation batch (≤20 samples)	% Recovery within project QC acceptance criteria	<ul style="list-style-type: none"> Reanalyze LCS Re-prep/reanalyze LCS and all associated samples Narrate all outliers
SW-846 8081A Organochlorine Pesticides by Gas Chromatography	Column Evaluation Mix	Prior to analysis, both initial and daily	Degradation of DDT and Endrin < 15%	<ul style="list-style-type: none"> Evaluate the system Repeat standard
	Initial calibration (5 point minimum) Lowest at or below PQL Mid level multi-component standards for pattern recognition and retention times	Prior to analysis and as required	RSD < 20%, average CF may be used; linear calibration required	<ul style="list-style-type: none"> Average RSD <20% across all analytes may be used if any analyte fails Evaluate the system Repeat initial calibration
	Initial calibration verification (ICV) Mid level standard Expected multi-component compounds	Prior to each 12 hour shift	% Difference ≤15% of expected concentration compared to response from ICAL	<ul style="list-style-type: none"> Average % difference ≤15% across all analytes may be used if any analyte fails Evaluate system/standard Reanalyze ICV standard Repeat initial calibration
	Continuing calibration verification (CCV) Mid level standard Expected multi-component compounds	After every 20 samples and at the end of the analytical sequence	% Difference ≤15% of expected concentration compared to response from ICAL for each bracketing standard	<ul style="list-style-type: none"> Average % difference ≤15% across all analytes may be used if any analyte fails Evaluate system/standard Reanalyze CCV and affected samples For CCV with response > initial calibration response and % difference >15%, samples need not be reanalyzed if no target compounds are detected
SW-846 8081 Organochlorine Pesticides by Gas Chromatography	Retention time windows	Established with each new column installation Updated with each daily initial calibration standard	Retention times must be within retention time window established by the daily initial calibration standard Every CCV and every sample	<ul style="list-style-type: none"> Evaluate system/standard; pattern recognition may be sufficient for multi-component compounds only Reanalyze CCV/affected samples

Analytical Method ^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
SW-846 8081 Organochlorine Pesticides by Gas Chromatography	Method Blank	1 per preparation batch (≤ 20 samples)	< ½ x PQL	<ul style="list-style-type: none"> Reanalyze blank Re-prep/reanalyze blank and associated samples
	Surrogate spike DCB and TCMX	Every sample, method blank, LCS and MS/MSD	% Recovery within QC acceptance criteria. One surrogate must fall within established control limits	<ul style="list-style-type: none"> Re-extract/reanalyze once If still out, report both sets of data Narrate all outliers
	Matrix spike (MS)	1 per preparation batch (≤20 samples)	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> Assess data (4 x rule) If LCS and surrogate recoveries are within acceptance criteria, matrix interference maybe suspected Re-extract/reanalyze once if matrix is not a factor Narrate all outliers
	Matrix spike duplicate(MSD) or Matrix Duplicate (MD)	1 per preparation batch (≤20 samples)	% Recovery and/or RPD within QC acceptance criteria.	<ul style="list-style-type: none"> Same as MS
	Laboratory control sample (LCS)	1 per preparation batch (≤ 20 samples)	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> Reanalyze LCS Re-prep/reanalyze LCS and all associated samples Narrate all outliers
SW-846 8151A Organochlorine Herbicides and Pentachlorophenol by Gas Chromatography	Initial calibration (5 point minimum) Lowest point at or below PQL	Prior to analysis and as required	%RSD <20%, average CF may be used; linear calibration required	<ul style="list-style-type: none"> Average RSD <20% across all analytes may be used if any analytes fail Evaluate the system Repeat initial calibration
	Initial calibration verification (ICV) second source Mid level standard	Prior to each daily analytical sequence	% Difference ≤15% of expected concentration compared to response from ICAL	<ul style="list-style-type: none"> Average %D ≤15% across all analytes may be used if any analytes fail Evaluate system/standard Reanalyze ICV standard Repeat initial calibration
	Continuing calibration verification (CCV) Mid level standard	After every 20 samples and at the end of the analytical sequence	% Difference ≤15% of expected concentration compared to response from ICAL for each bracketing standard	<ul style="list-style-type: none"> Evaluate system/standard Reanalyze CCV and all samples back to last acceptable CCV
	Retention time windows	Established with each new column installation Updated with each daily initial calibration standard	Retention times must be within retention time window established by the daily initial calibration standard Every CCV and every sample	<ul style="list-style-type: none"> Evaluate system/standard; Reanalyze CCV and affected samples
	Method blank	1 per preparation batch (≤20 samples)	< ½ x PQL	<ul style="list-style-type: none"> Reanalyze blank Re-prep/reanalyze blank and all associated samples
	Surrogate spike DCAA	Every sample, method blank, LCS and MS/MSD	% Recovery within project QC acceptance criteria	<ul style="list-style-type: none"> Re-extract/reanalyze once If still out, report both sets of data Narrate all outliers

Analytical Method^(a)	QC Element	Frequency	Acceptance Criteria	Corrective Action
SW-846 8151A Organochlorine Herbicides and Pentachlorophenol by Gas Chromatography	Matrix spike (MS)	1 per preparation batch (≤20 samples)	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> • Assess data (4x rule) • If LCS and surrogate recoveries are within acceptance criteria, matrix interference maybe suspected • Re-exact/reanalyze once if matrix is not a factor • Narrate all outliers
	Matrix spike duplicate (MSD) or Matrix duplicate (MD)	1 per preparation batch (≤20 samples)	% Recovery and/or RPD within QC acceptance criteria	<ul style="list-style-type: none"> • Same as MS
	Laboratory control sample (LCS)	1 per preparation batch (≤20 samples)	% Recovery within QC acceptance criteria	<ul style="list-style-type: none"> • Reanalyze LCS • Re-prep/reanalyze LCS and all associated samples • Narrate all outliers

EICP Extracted ion current profile
 QC Quality control
 RF Response factor
 RSD Relative standard deviation

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Table 12-1 General Evaluation Methods for Assessing Asbestos Data Usability

Data Usability Indicator	General Evaluation Method
Precision	<p><u>Sampling</u> – Review results for field duplicates to provide information on variability arising from medium spatial heterogeneity and sampling and analysis methods.</p> <p><u>Analysis</u> – Review results for PLM laboratory duplicates, TEM recounts, and TEM reparations to provide information on variability arising from analysis methods. Review results for inter-laboratory analyses to provide information on variability and potential bias between laboratories.</p>
Accuracy/Bias	<p>TEM – Calculate the background filter loading rate and use results to assign detect/non-detect in basic accordance with ASTM 6620-00.</p> <p>PLM – Review results for LA-specific performance evaluation standards to provide information on direction/magnitude of potential bias.</p>
Representativeness	Review relevant field audit report findings and any field/laboratory ROMs for potential data quality issues.
Comparability	Compare the sample collection SOPs, preparation techniques, and analysis methods to previous investigations.
Completeness	Determine the percent of samples that were able to be successfully collected and analyzed (e.g., 99 of 100 samples, 99%).
Sensitivity	TEM – Determine the fraction of all analyses that stopped based on the area examined stopping rule (i.e., did not achieve the target sensitivity).

ASTM = American Society of Testing and Materials

LA = Libby amphibole

PLM = polarized light microscopy

QATS = Quality Assurance Technical Support

ROM = record of modification

SOP = standard operating procedure

TEM = transmission electron microscopy

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Appendices

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Appendix A

Study Protocols

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APPENDIX A.1
Protocol for the Amphibian Laboratory Toxicity Test
(Revision 0 – April 20, 2012)

FEL Protocol No.: **GOLD03-1**

Study Title: **Amphibian Complete Metamorphosis Exposure Study**

Test Guideline: **Libby Asbestos Superfund Site Phase V-B SAP, OU 3**

FEL Study No.: **GOLD03-00277**

Proposed Start Date: **April 2012**

Test System: ***Rana sp.***

Test Article: **Libby Amphibole Asbestos (LA)**

Sponsor: **Golder Associates, Inc.
18300 NE Union Hill Road, Suite 200
Redmond, WA 98052**

Golder Associates Project No. 103-93351

Study Facility: **Fort Environmental Laboratories, Inc. (FEL)
515 South Duncan Street
Stillwater, OK 74074**

Analytical Lab (LA): **EMSL Analytical, Inc. (EMSL)
107 West 4th Street
Libby, MT 59923**


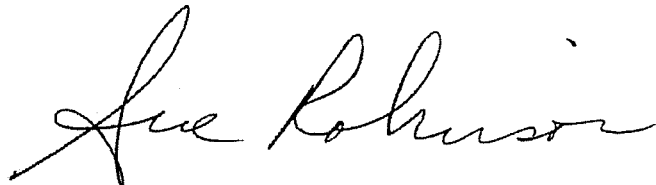
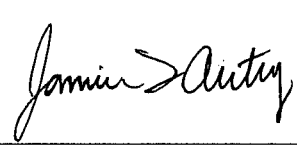

Preparation Facility (LA): **EPA Sample Preparation Facility
303 N. 3rd Street
Troy, MT 59935**

**Analytical Lab:
(Other Contaminants)** **Energy Laboratories
1120 South 27th Street (59101)
Billings, MT 59107-0916**

Amendments:

Number	Date	Section(s)	Page(s)
1			
2			
3			
4			

1. SIGNATURE PAGE

Title/Name	Signature	Date
STUDY DIRECTOR: Douglas J. Fort, Ph.D.		4/20/12
SPONSOR REPRESENTATIVE: ¹ Sue Robinson Golder Associates, Inc.		4/20/12
STUDY LEAD: Jamie Autry		4/20/12
QAU MANAGER: Michael Mathis		4/20/12

¹Study Monitor.

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2. QUALITY ASSURANCE

This study will be conducted “in the essence” of Good Laboratory Practices (GLP) principles, but is not intended to directly comply with all intended requirements of the GLPs. Fort Environmental Laboratories, Inc. (FEL) Quality Assurance Unit (QAU) will provide a Quality Assurance (QA) Statement in the final report certifying review of the data and report. This statement will be signed by the responsible QAU.

3. INTRODUCTION

FEL has been contracted by Golder Associates (Golder) to conduct a toxicological study that will examine the effects of Libby Amphibole asbestos (LA) on the complete metamorphosis of ranid amphibians. The study will be used in support of the evaluation of potential ecological risk at the Libby Asbestos Superfund Site, in Libby, Montana. The study will be conducted in accordance with the specifications identified in the Phase V-B Sampling and Analysis Plan (SAP) (1), FEL Quality Assurance Management Plan (QAMP) (2), relevant facility standard operating procedures (SOPs), and Study Protocol No. GOLD03-1 prepared for FEL Study No. GOLD03-0277. The analysis of the resulting bioassay data (statistics) and reporting will also be in accordance with any Phase V-B SAP requirements (1).

4. OBJECTIVE

A complete amphibian metamorphosis assay will be performed in which ranid larvae (ca. Gosner stage 20) (3) are exposed to LA. The general experimental design will entail exposing tadpoles to: 1) laboratory dilution water and inert sterilized sand, 2) laboratory dilution water and reference sediment, and 3) laboratory dilution water and field-collected sediment from the Libby site containing approximately 2% LA. Larval density at test initiation will be 20 tadpoles per test tank. Four replicates will be evaluated for each treatment group or control (80 organisms exposed per treatment or control). The treatment tanks will be randomly assigned to a position in the exposure system in order to account for possible variations in temperature and light intensity. The primary endpoints will be survival, developmental stage, time to metamorphosis (TTM) for each specimen, the median time to metamorphosis (MMT) for each replicate and treatment, metamorphic count, external and internal malformations, whole body weight, and snout-vent length (SVL) in each surviving specimen. These endpoints are included in the present study because they could reflect potential effects on the population of amphibians within the study site. Effects on SVL and external malformations could potentially affect survival. In addition, malformations could also affect growth/metamorphosis and possible reproductive performance. Blood, head (thyroid), and torso (containing presumptive gonad) will be preserved for possible further study, if deemed necessary based on the results of this study (GOLD03-00277), but such further study is not within the scope of present study.

5. STUDY FACILITIES

The in-life portion of the study, to include the pre-exposure equilibration period and exposure phases of the amphibian metamorphosis study, will be performed at Fort Environmental Laboratories, Inc., 515 South Duncan Street, Stillwater, OK, USA, 74074. The Study Director, Dr. Douglas Fort, will serve as the study contact for this facility and may be reached at 405.624.6771 or djfort@fortlabs.com.

The LA analysis of sediments will be performed at EMSL (Libby, MT). Ron Mahoney, Principal Investigator (PI) of the planned analyses, will serve as study contact for EMSL and may be reached at 406.293.9066 or Rmahoney@EMSL.com. Prior to analysis, sediment samples will be prepared (dried, sieved, ground) at the EPA Sample Preparation Facility (SPF) in Troy, MT. Andrea Wandler (TechLaw, Inc.) will serve as study contact for the SPF and may be reached at 406.295.9151 or awandler@techlawinc.com.

Other contaminant analyses of the target site (TP-TOE2 and Carney Creek) sediments will be performed at Energy Laboratories (Billings, MT). Bill Brown, Principal Investigator (PI) of the planned analyses, will serve as study contact for Energy Laboratories and may be reached at 406-252-3265 ext. 6270 or bbrown@energylab.com.

6. ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor will make particular note of the following:

- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress, or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
- By design, this study may kill and/or result in the pain and distress of test organisms. Euthanasia of test organisms before completion of the test would interfere with study objectives. Upon completion of the test, all distressed amphibians will be painlessly euthanized in a timely manner.
- Methods of euthanasia used during this study are in conformance with the above referenced regulation.

7. STUDY SCHEDULE

The experimental study is proposed to start in the spring of 2012 after Sponsor (and USEPA) approval of the Final Study Protocol. Although the median developmental time to complete metamorphosis is 45-60 days, the maximum duration will be set at 90 days. Therefore, the actual in-life test duration is estimated to be no greater than 90 days. The experimental study termination is anticipated to occur after completion of the necropsy.

8. EXPERIMENTAL DESIGN

Experiment design will follow the general methods established in the Phase V-B SAP for OU3 (1).

8.1. Test Substance

The test substance will be natural LA contained within site sediment. Test chambers will be 2.5-gallon glass aquaria containing 1.5 Kg sediment and 6 L of laboratory dilution water. The three treatments will consist of: 1) laboratory dilution water and inert sterilized sand, 2) laboratory dilution water and reference sediment, and 3) laboratory dilution water and field-collected sediment from the Libby site containing approximately 2% LA. Each treatment will have four replicates with 20 larvae per replicate (n = 80 per treatment). Exposure will span

young newly free swimming larvae (Gosner stage 20) through complete metamorphosis. Water will be renewed using a flow through system. Sediment will not be renewed. Overlying water within the chambers will be aerated using a micro-bubble diffuser to provide sufficient aeration if needed. All construction and materials in contact with test water will be glass, stainless steel, or Teflon®. Sediment collected by Remedium (on-site, Libby, MT) at the Libby mine location (i.e., TP-TOE2 or Carney Creek) containing ca. 2% LA will be used for exposure #3 (laboratory dilution water and site sediment). Sediment collection methods are as outlined in the Phase V-B SAP (1). Uncontaminated field reference sediment (exposure #2) will be collected by FEL from a reference pond in Oklahoma and will be of similar constitution as the test sediment collected at the mine site. Laboratory control (synthetic) sediment will consist of inert sterilized sand (exposure #1). Both the reference sediment and lab control sediment (sand) will be analyzed for contaminants (total metals, polyaromatic hydrocarbons [PAHs], and organochlorine pesticides/polychlorinated biphenyls [PCBs as Aroclors]). In addition, sediment collected from site TP-TOE2 and alternate site Carney Creek will be analyzed for LA (EMSL, Libby, MT), organochlorine pesticides (SW 8081A), chlorinated herbicides (SW 8151A), TOC (ASA 29-3), diesel and gasoline range organic (SW 8015B), Hg (SW7471A), total metals (E6010.20), moisture (D2974), pH (ASA 10-3), PCBs (Aroclors, SW 8082), semi-volatile organics (SW 8270C), acid volatile sulfide (AVS/TTR), and ammonia (as N) (ASA 33-7) (other noted contaminants above will be measured by Energy Labs, Billings, MT) prior to use. LA will be measured again at the conclusion of the treatment exposure to verify exposure concentrations. In the event that TP-TOE2 contains lower levels of LA based on analyses of the sediment, an alternative site will be selected. The alternate site will be Carney Creek. The in-life study will not be initiated until analytical results confirming the LA contamination levels in the site sediment, or the lack of LA contamination in the lab control sediment or reference sediment. At exposure termination, sediment will be collected from each replicate tank, water decanted, and the sediment shipped to the SPF in Troy, MT for preparation prior to LA analysis by EMSL (Libby, MT).

8.2. Dilution and Laboratory Control Water

FEL will use sterilized dechlorinated (charcoal-filtered) tap water as the dilution water for this study. The dechlorinated laboratory water will be prepared by passing tap water through a 4 filter system; a multimedia filter to remove suspended solids in the feed water; a 10" pre-treatment filter (5 µm) to remove any additional solids; a 3.6 ft³ activated virgin carbon treatment filter to remove chlorine, ammonia, and higher molecular weight organics; and a 5 µm polishing filter to remove any carbon particles from the carbon treatment phase. The sterilized dechlorinated tap water will also serve as the laboratory control water. Water quality characteristics of the laboratory water are monitored bimonthly for pH, dissolved oxygen (DO), conductivity, hardness, alkalinity, ammonia, residual oxidants; and at least annually for iodide, polyaromatic hydrocarbons (PAHs), pesticides, and metals. The dilution water was most recently analyzed for pesticides, PAHs, and metals on January 20, 2012 and all water quality measurements cited above met the U.S. EPA and American Society for Testing and Materials (ASTM) criteria for aquatic toxicity test culture/culture water (5,6).

Sufficient iodine needs to be available to the larvae through a combination of aqueous and dietary sources for the thyroid gland to synthesize thyroid hormones to support normal metamorphosis. Because of this, use of sterilized dechlorinated tap water rather than

reconstituted water without iodide is important for the successful completion of this study. If the iodide concentration in the dilution water is relatively consistent (coefficient of variance [CV] \leq 20%), measurement of aqueous iodide concentrations from the dilution water can be measured at least once per year and reported with the study data. Based on previous work (4,6), the amphibian metamorphosis assay has been demonstrated to work well when test water iodide concentrations ranged between 0.5 and 15.0 $\mu\text{g/L}$. The dilution water at FEL was analyzed most recently on January 16, 2012, and contained 11.3 $\mu\text{g/L}$ iodide, which falls within the acceptable range.

8.3. Test System

The test species will be a ranid to be determined based on availability from which young free-swimming larvae (Gosner stage 20 [3]) will be used in the complete metamorphosis assay. Ranids are well-studied anuran amphibians that exhibits ease of use in the laboratory, rapid development, and simplicity of observation.

8.3.1. Origin, Handling, and Feeding

The ranid larvae used for this study will be obtained from eggs collected from a commercial source and will be one of the following species: southern leopard frog (*Rana sphenoccephala*), northern leopard frog (*Rana pipiens*), green frog (*Rana clamitans*), or wood frog (*Rana sylvatica*). Northern leopard frog will be the preferred species followed by the southern leopard frog and the green frog. However, any one of these species will produce acceptable results. Alternative species may be considered based on season with the exception of bull frogs (*Rana catesbeiana*). The source of the study specimens will be specifically documented in the study documentation and final report. Details for larval care, specific to FEL, are found in SOP 8.2 (5). All tadpoles that are used as test organisms will be derived from the same clutch (spawn). Embryos will be held at 22-24°C for 7 d to allow for hatching. After hatching, larvae will be separated to a density of 5-10 animals/L at a constant flow rate (12 mL/minute) and water temperature (20-23° C) under these physicochemical conditions until they reach Gosner stage 20.

Tadpoles will be fed boiled organic romaine lettuce leaves *ad libitum*. Lettuce leaves are a standard diet used for most omnivorous amphibian larvae with the exception of *Xenopus* sp. (5,6). Lettuce will be used *in lieu* of Sera Micron® (commonly used for *Xenopus* sp.) to minimize biofouling of the tank water. Sera Micron is a highly concentrated nutrient-rich algae suspension which is dense and has the propensity to initiate algal growth in tanks in which it is used. Biofouling was shown in earlier pilot studies to alter fiber bioavailability. FEL has successfully cultured ranid and bufonid species using boiled romaine lettuce leaves. Boiling the lettuce breaks down the cellulose in the lettuce which makes the lettuce easier to digest and reduces the degradation process in the leaf which could cause biofouling of the water. Documentation of the amount of lettuce consumed by the test specimens will be recorded by weighing the lettuce after boiling before introduction to the tank and recording weight after removal of any intact waste lettuce.

8.4. Exposure System

The media for exposure will be sediment. Exposure will be maintained using a flow-through system in which culture water, but not sediment, will flow through the tanks at a rate of 12/mL/min (2.9 volume exchanges/day). Sediment and sand (1.5 Kg) will be added directly to the bottom of each appropriate aquarium and 6 L of dilution water added (1:4 ratio). The sediment/sand and water will be allowed to equilibrate for 24 hr prior the introduction of test organisms. Daily cleaning of the tanks using a turkey baster will remove organism detritus and excrement from the bottom of the tanks. This will help minimize bio-fouling and will help maintain water quality. Care will be taken not to disturb the sediment. Exposure tanks will be glass aquaria (with approximate measurements of 22.5 x 14.0 x 16.5 cm deep) equipped with standpipes that result in an actual tank volume of 6 L, with 2 cm of sediment, and minimum water depth of 25-30 cm. Fluorescent lighting will be used to provide a photoperiod of 12 hours (hr) light and 12 hr dark at an intensity that ranges from 600 to 2,000 lux (lumens/m²) at the water surface. Water temperature will be maintained at 20-23°C, pH maintained between 6.5 to 8.5, and the dissolved oxygen (DO) concentration > 3.5 mg/L (> 40% of the air saturation) in each test tank.

8.5. Water Quality and Sediment Analyses

8.5.1. Dilution Water Quality Analyses

In each replicated tank, temperature will be measured daily; and pH, DO, and light intensity (lux) will be measured three times per week. Total hardness, alkalinity, conductivity, total residual oxidants, and ammonia-nitrogen will be measured in the each replicate of each treatment at the beginning and conclusion of the in-life exposure, and semimonthly during the exposure phase prior to renewal.

8.5.2. Sediment Analysis for LA

Test sediment (treatment group 3) will be analyzed for LA in accordance with the Libby-specific polarized light microscopy visual area estimation (PLM-VE) method by EMSL (Libby, MT)¹. Sediment used for treatment group 3 will be collected in several “lots” by Remedium at TP-TOE2 and Carney Creek. Each lot will be well mixed prior to analysis. Sediment lots will be analyzed following the Libby-specific PLM-VE methods and the sediment lot(s) with concentration at 2% (or higher) submitted to FEL for testing. At the conclusion of the study, each replicate of the three treatments will be gently decanted to remove overlying and excess water. A sample of the wet sediment from each replicate will be collected in a glass bottle and submitted to the SPF in Troy, MT for preparation and subsequent analysis by EMSL (Libby, MT) to determine the final test exposure concentrations for treatment 3. Only sediment (treatment exposure medium) will be analyzed for LA in the present study (i.e., the overlying water will not be analyzed for LA).

8.5.3. TP-TOE2 (or Carney Creek) Sediment Analysis for Other Contaminants

In order to determine other contaminants within the TP-TOE2 (or Carney Creek) sediment that could potentially affect the study, an additional aliquot of the sediment sample

¹ Samples will be prepared prior to analysis by the SPF in Troy, MT.

collected from TP-TOE2 and Carney Creek for the bioassay will be submitted to Energy Labs (Billings, MT) for analysis of the contaminants described for the target site in Section 8.1 prior to initiation of the bioassay. The methods to be used are also cited in Section 8.1. Energy will report the results of these analyses to the Study Sponsor and FEL.

8.6.Equipment

The following equipment will be needed:

- Study book (daily observations and data recording)
- Glass aquaria;
- UV sterilization tubes and 0.2 µm bacteriostatic filters (as needed);
- Aquarium heaters (adjustable to 20-23°C);
- Thermometer;
- Binocular dissection microscope;
- Digital camera with at least 4 mega pixel resolution and macro-function (general test photo documentation, test termination for digitizing and documentation);
- Image digitizing software;
- Petri dish (100 x 15 mm) or transparent plastic chamber of comparable size;
- Analytical balance capable of measuring to 3 decimal places (mg);
- Dissolved oxygen meter;
- pH meter;
- Light intensity meter capable of measuring in lux units;
- Miscellaneous glassware (beakers, volumetric flasks, Erlenmeyer flasks, graduated cylinders, etc.);
- Adjustable pipettors (10 to 5,000 µL) or assorted pipettes of equivalent sizes;
- Top stirrer;
- Sample collection bottles;
- Water baths; and
- Turkey basters

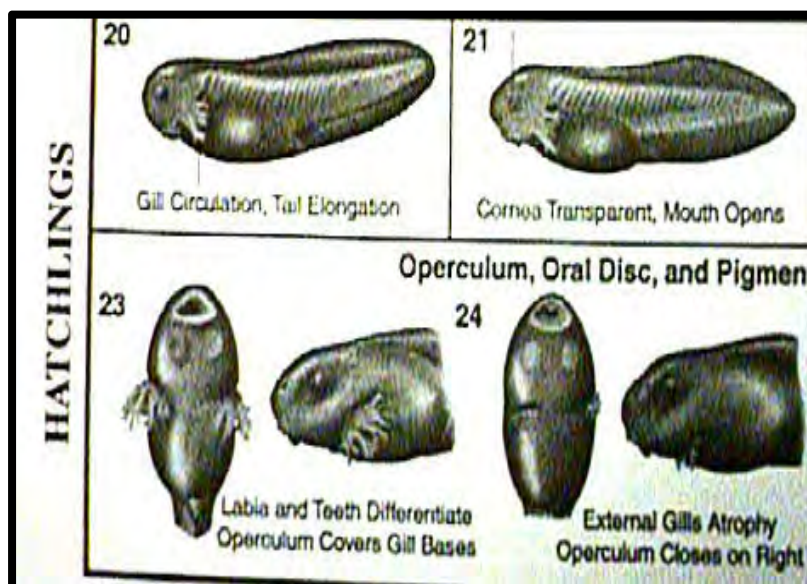
8.7.Test Animal Selection

When a sufficient number of the pre-exposure population reach Gosner (3) developmental stage 20 (ca. 1 d post-hatch) (see Figure 1), larvae will be transferred to a pooling tank containing dilution water. General requirements for determining acceptable organism development will need to be used since the specific ranid species to be used cannot be determined until the protocol is finalized and study schedule specifically set. Larvae requiring >5 d post-hatch to reach Gosner stage 20 will not be used in the in-life test. If >50% of the larvae in a given clutch require >20 d to reach Gosner stage 20, an alternate clutch will be used. All larvae used in the in-life study will be from the same clutch of offspring. Individual larvae will be randomly removed from the pooling tank by scooping with a small strainer. Animals will be carefully handled during this transfer in order to minimize handling stress and to avoid any injury.

The developmental stage of the animals will then be determined by using a binocular dissection microscope. The primary developmental landmark for selecting Gosner stage 20 organisms is tail morphology (3).

Animals that meet the stage criteria will be transferred to a holding tank containing 100% dilution water. The selected larvae will be randomly distributed to exposure treatment tanks (including the control) containing 6 L of treatment solution until each tank contains 20 larvae. The standard test organism density requirement for amphibians is 0.5 g tissue/L (6). Larvae typically weigh 100-200 mg. Therefore, the average initial biomass in each tank will be ca. 0.5 g/L (150 mg/organism x 20 organisms/tank). Each treatment tank will then be inspected for animals with abnormal appearance (e.g., injuries, abnormal swimming behavior, etc.). Overtly unhealthy looking tadpoles will be removed from the treatment tanks and replaced with larvae newly selected from the holding tank. Treatment tanks will be labeled with the study, treatment, and replicate identification at a minimum. The treatment tanks will be randomly assigned to a position in the exposure system in order to account for possible variations in temperature and light intensity.

Figure 1. Gosner stage 20



8.8. Test Method

The randomly selected Gosner stage 20 larvae will be assigned to one of the five treatments:

- 1) laboratory dilution water and inert sterilized sand,
- 2) laboratory dilution water and reference sediment, and
- 3) laboratory dilution water and field-collected sediment from the Libby site containing approximately 2% LA.

Each exposure treatment and control/reference will be evaluated in quadruplicate (i.e., 4 replicates), with 20 organisms per replicate. Once all larvae have been assigned to an exposure system, mortality observations and developmental stage determination will be made daily and any dead larvae will be immediately removed, preserved in 10% neutral buffered formalin (NBF), and necropsied. During the exposure phase, the number of organisms metamorphosed will be recorded as will the time to metamorphosis (TTM) for each larvae, the weight of each newly metamorphosed larvae, and the median time to metamorphosis (MMT) determined when 50% of the larvae in a given replicate metamorphose. The exposure phase will be terminated when each of the surviving control (treatment #1) larvae complete metamorphosis. Upon exposure termination, test organisms will be anesthetized in 150 mg/L 3-aminobenzoic acid ethyl ester (MS-222) (pH 7) digital photos will be taken to measure SVL, whole body weight measured, external malformation assessed, and blood collected. The test organisms will then be euthanized using 200 mg/L MS-222 and visceral (internal) abnormalities assessed in the completely metamorphosed specimens. The head and carcass (with gonads) will be fixed in Davidson's Solution and preserved in 10% NBF for possible future histopathology, if warranted, which would be conducted separately from project GOLD03-00277. Critical test parameters and experimental conditions for the in-life study are presented in Table 1.

8.9.Data Collection and Biological Endpoints

Test data and daily observations will be recorded in the study records. Study records will include study tracking sheets, test information sheets, study calendars identifying major events, study logs for recording detailed observations and comments, daily mortality and developmental stage data sheets, test termination data sheets, and representative digital photographs taken during the conduct of the test. These photographs will document the study design, study milestones, and endpoints (length measurements, development (external), abnormalities, and necropsy of the fully metamorphosed specimens. The primary endpoints of the metamorphosis assay will be mortality; developmental stage (Gosner); number of specimens metamorphosed, weight at metamorphosis; TTM (and MMT for each replicate); snout-vent length (SVL) with digital photographs; wet whole body weight; and external and internal abnormalities (documented with digital photographs for inclusion in the study records). Table 2 provides an overview of the measurement endpoints and the corresponding observation time points.

8.9.1. Mortality

All test tanks will be checked daily for dead larvae and the numbers will be recorded for each tank. Dead animals will be removed from the test tank as soon as observed. Mortality rates exceeding 20% may indicate inappropriate test conditions or toxic effects of the test material. Excessive mortality occurring within the first several weeks of exposure will be reported directly to the Sponsor for discussion with USEPA including any further instructions on the conduct of the study.

8.9.2. Developmental Stage

The developmental stage of the tadpoles will be determined by using the staging criteria of Gosner (3). Developmental stage data will be used to determine if development is accelerated, asynchronous, delayed or unaffected. Acceleration or delay of development is determined by making a comparison between the median stage achieved by the control and

treated groups. Asynchronous development will be reported when the tissues examined are not malformed or abnormal, but the relative timing of the morphogenesis or development of different tissues is disrupted within a single tadpole. For the purposes of generally tracking development throughout the exposure phase (laboratory purposes only, not for presentation or further analysis) an estimated field stage within a given replicate will be determined daily through metamorphosis.

8.9.3. Metamorphic Completion, Metamorphosed Weight, Time to Metamorphosis

The number of live tadpoles completing metamorphosis in each replicate of each treatment will be determined. Upon metamorphosis, tadpoles will be anesthetized in 200 mg/L MS-222 (pH 7) and weighed. The TTM in days will be determined for each individual frog and the MMT determined for each replicate of each treatment or control.

8.9.4. Body Length (SVL)

SVL will be the first of two endpoints used to assess growth in anesthetized frogs. All length measurements (mm) will be based on digital photographs of the surviving organisms from each treatment. SVL will be evaluated at in-life test termination immediately prior to necropsy.

8.9.5. Wet Body Weight

Determinations of wet body weight (nearest mg) will be used to assess possible effects of LA on the growth rate relative to the control and reference group in anesthetized frogs. Wet weight measurements will be performed on surviving organisms at in-life study test termination immediately prior to necropsy.

8.9.6. External and Internal Abnormalities

While developmental stage, metamorphic completion, and TTM are important endpoints to evaluate exposure-related changes in metamorphic development and SVL and body weight are indicators of growth, the most profound effects are abnormal development. External and internal (visceral) morphology will be evaluated in all surviving specimens at the conclusion of exposure to determine if exposure to LA is capable of inducing abnormal development. External abnormalities will focus on eyes, mouth, torso, and hind limbs. Internal abnormalities will focus on primary organ systems, such as liver, kidneys, heart, and lung. Digital photographs of all abnormalities observed will be taken, with the organism treatment designation and replicate number visible, to support the study record. Although the gonad will not have fully differentiated at the conclusion of the study, the presumptive gonad tissue (primary differentiation) will be present. Head and carcass samples from the euthanized specimens will be fixed in Davidson's solution and preserved in 10% NBF for possible future histopathological examination of the thyroid gland and presumptive gonad tissue (both under separate study), if warranted based on the results of the current study.

8.9.7. Additional Observations and Measurements

All cases of abnormal behavior (e.g., uncoordinated swimming, hyperventilation, atypical quiescence, non-feeding, etc.) will be recorded in the study records and included in the final study report. Representative digital photographs will be taken for the study record of abnormal behavior to the extent possible. Plasma will be collected from MS-222 anesthetized specimens

and stored frozen for possible future thyroid hormone measures (separate study), if warranted based on the results of the current study (7).

8.10. Day 0 Test Initiation and Sample Collection

On study day 0, healthy and normal looking tadpoles of the stock population will be pooled in a single vessel containing an appropriate volume of dilution water. For developmental stage determination, tadpoles will be individually removed from the pooling tank using a small net or strainer and transferred to a transparent measurement chamber containing dilution water. No anesthesia will be used. Animals will be carefully handled during this transfer to minimize handling stress and to avoid injury. The developmental stage of the animals will be determined using a binocular dissection microscope.

Tadpoles that meet the stage criteria described above in this protocol will be held in a tank of clean culture water until the staging process is completed. Once the staging is completed, the larvae are randomly distributed to exposure treatment tanks until each tank contains 20 larvae. Each treatment tank is then inspected for animals with abnormal appearance (e.g., injuries, abnormal swimming behavior, etc.). Overtly unhealthy tadpoles will be removed from the treatment tanks and replaced with larvae newly selected from the pooling tank. Five randomly selected Gosner stage 20 pre-exposed tadpoles will be humanely euthanized in 150 to 200 mg/L MS-222 (pH 7) and preserved to verify stage upon in-life test setup.

8.11. Measurements at Metamorphosis

At metamorphosis, organisms will be removed from each test tank and anesthetized in 150 to 200 mg/L MS-222. The frogs will be rinsed in water and blotted dry, followed by body weight determination to the nearest mg. The total number of organisms reaching metamorphosis, the TTM for each individual, and the MMT for each replicate of each treatment will be determined.

8.12. Measurements at Test Termination

When all of the surviving control (treatment #1) larvae complete metamorphosis, exposure, exposure will be terminated in all treatments. At test termination, specimens will be removed from the test tanks anesthetized in 200 mg/L MS-222, weighed, and digitally photographed to determine SVL. Plasma samples (7) will then be collected and external malformations scored and recorded with digital photographs. The organisms will then be humanely euthanized in 200 mg/L MS-222 and necropsied. External and internal abnormalities will be determined and presumptive gonad tissue identified. The head and carcass (with presumptive gonad tissue) will then be fixed in Davidson's solution and subsequently preserved in 10% NBF for possible histopathological examination (separate study), if warranted based on the results of the current study.

Prior to fixing tissue for histopathological analyses, blood samples will be collected from each buffered MS-222 anesthetized larvae, plasma isolated, and stored for possible future analysis of thyroid hormone (thyroxine [T4]) in accordance with FEL SOP (8), if warranted based on the conclusions from the present study. Plasma and retained tissues will be stored at -20°C. All blood and tissue samples will be stored frozen and analyzed at the discretion of the

Sponsor under a separate study. Head and carcass samples from all organisms will then be placed in Davidson's fixative for 48 to 72 hours as whole body samples for possible future histological assessments (separate project), if warranted based on the results of the current study. Specimens will then be rinsed in dechlorinated tap water and preserved in 10% (w/v) NBF (8).

8.13. Data Analysis and Statistics

All data from in-life portions of the study will be tabulated in spreadsheets by FEL. Statistical analyses of the data will generally follow procedures described in the document Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application (9). The precise statistical tests that will be used to compare exposed and control organisms will vary between the measurement endpoints. For discrete endpoints (survival, malformation frequency), it is expected that comparisons will be made using the Fisher Exact test. For continuous endpoints (body weight), it is expected that the comparisons between control and treated groups will be performed using the Wilcoxon Rank Sum (WRS) Test if non-normal and performed using t-statistics if normally distributed. A significant treatment effect for developmental stage will be determined on the replicate median values using a Mann-Whitney U test or WRS. In the event median values cannot be determined, replicate mean stage values will be used and evaluated using Dunnett's test. Other statistical tests that may be appropriate include one-way analysis of variance (ANOVA) or an ANOVA on ranks. *Post hoc* tests may also be used such as Dunnett's test or Bonferroni t-test for parametric sets, or Dunn's test for non-parametric tests. If these comparisons show significant differences or are inconclusive, additional histological investigations may be considered. To accommodate potential future histopathological and endocrinological investigations, the body (head and carcass) [histopathology] and blood plasma (thyroid hormone) will be preserved. If no statistically significant differences in any of the endpoints are detected between the exposed and the control organisms, it will be concluded that exposure to LA in sediment at concentrations equal to or less than the levels tested are not likely to cause effects that are ecologically significant. If statistically significant changes in one or more measurement endpoints are observed, additional investigation, including evaluation of preserved tissues, may be warranted to determine the subsequent steps.

Table 1. Experimental Conditions for In-Life Study

Test Article		LA in OU3 Sediment
Test System (species)		<i>Rana</i> larvae
Initial Larval Stage		Gosner Stage 20
Exposure Period		Complete metamorphosis (terminated when the treatment #1 controls complete metamorphosis)
Larvae Selection Criteria		Developmental Stage
Treatments and Controls		1) laboratory dilution water and inert sterilized sand, 2) laboratory dilution water and reference sediment, 3) laboratory dilution water and field-collected sediment from the Libby site containing approximately 2% LA.
Exposure System		Flow through (12 mL/min.)
Exposure Route		Sediment
Renewal Frequency		2.9 volumes/d
Primary Endpoints / Determination Days	Survival/Stage	Daily
	Metamorph Count/Weight	Cumulative/Individual Weight
	TTM/ MMT	Cumulative
	SVL	Termination
	Wet Body Weight	Termination
	External malformations	Termination
	Internal abnormalities	Termination
Additional Samples (stored for possible future analysis)		Plasma - TH, head and carcass – histopathology, if warranted based on results of current study
Dilution Water / Laboratory Control		Dechlorinated Tap Water
Larval Density		20 Larvae / Test Vessel (3.3/ L)
Test Solution / Test Vessel		6 L (ca. 20 cm water height)
Replication		4 Replicates/Exposure treatment
Acceptable Mortality Rate in Controls		≤20%
Media Parameters	Temperature	20-23°C
	pH	6.5-8.5 su
	DO	>3.5 mg/L
Feeding	Food	Boiled romaine lettuce
	Frequency / Amount	Twice daily M-F (1x S S) / see Table 1, ad libitum, and 1x MWF
Lighting	Photoperiod	12 h light : 12 h dark
	Intensity	600 to 2,000 lux (Measured at Water Surface)
Analytical Chemistry Sample Schedule		Sediment – T0 and at in-life phase conclusion

Table 2. Observation Time Points for Primary Effect Measures

Apical/Molecular/Biochemical Endpoints:	Daily	Prior to Necropsy	At Necropsy
Survival:			
Mortality	•		
Malformation		• ¹	
Growth:			
Whole body weight		•	
SVL		•	
Malformation		• ¹	
Metamorphosis:			
Developmental stage		•	
TTM and MMT		•	
External malformations			• ¹
Internal abnormalities			• ¹
Additional (tissues collected only)²:			
Plasma		•	
Head and carcass			•

¹ Digital photographs of potentially significant malformations/abnormalities

² Collected for analysis if warranted based on the results of the present study.

8.14. Performance Criteria and Test Validity

General test performance criteria are provided in Table 3.

Table 3. General Test Performance Criteria

Criterion	Acceptable Limits (10)
Control mortality	≤ 20% in any replicate of the control
Maximum MMT	90 d
Range of control MMT	≤ 4 for the 10 th and 90 th percentile
DO	> 40% of air saturation
pH	6.5 – 8.5 s.u.
Water temperature	20 -23°C with inter-replicate variability ≤ 1.0°C
Replicate performance	≤ 2 replicates amongst test can be compromised

9. SAMPLE HANDLING AND CUSTODY

All samples received (11) and generated for testing in this study (sediment samples at conclusion of in-life exposure) will be accompanied with an appropriately signed chain of

custody when sent to the SPF in Troy, MT and handled in accordance with facility SOPs (12). Samples received for testing will be entered into a sample check-in logbook and assigned a unique sample tracking number to FEL. FEL samples numbers consist of the client number (GOLD01), the project number (00227), and the sample identifier (00XXX) (12). Each sample will also be properly labeled with its assigned sample tracking number. Samples, when not in use, will be properly preserved and stored, based on sample matrix.

10. REPORTING

FEL will provide progress reports to the Study Monitor throughout the actual study, as required. The Study Monitor will visit FEL during the conduct of the study to observe the study and document protocol compliance. At study conclusion, a Final Report will be provided to the Sponsor by FEL. The report will include the following:

Study report will include:

- **Executive Summary**
- **Test Article:**
 - Test article: Will include name and identifiers if applicable.
 - Chemical observations and data: Will include method and frequency of preparation of stock solutions, nominal and measured concentrations of the test chemical, and in some cases, non-parent chemical, as appropriate.
- **Test System:**
 - Organism: Will include scientific name, age, supplier, pre-treatment (if used)
- **Test conditions:**
 - Test method: Will include range-finding design and definitive test design, delivery process (static-renewal), aeration, test system loading
 - Operational parameters records: These parameters will consist of observations pertaining to the functioning of the test system and the supporting environment and infrastructure. Records will include: ambient temperature, test temperature, photoperiod, status of critical components of the exposure system (e.g., pumps), flow rates, water levels, stock bottle changes, and feeding records. General water quality parameters will include: pH, DO, conductivity, total iodine, alkalinity, hardness, ammonia-nitrogen, and total residual oxidants.
 - Analytical methods: Analytical methods used by EMSL (Libby, MT) for measurement of LA in sediment will be included in the report.
 - Deviations from the test method: This information will include any information or narrative descriptions of deviations from the test methods and procedures established in this protocol.
- **Results:**
 - Biological observations and data: Information will include daily observations of mortality, food consumption, abnormal swimming behavior, lethargy, loss of equilibrium, malformations, lesions, TTM, and MMT. Observations and data collected at predetermined intervals include:

developmental stage, hind limb length, SVL, and wet body weight. Representative digital photographs will be taken to document the conduct of the study.

- Analytical results: Results of analytical testing of LA in sediment by EMSL (Libby, MT) and evaluation of analytical results in relation to the relevant test acceptance criteria will be included in the report.
- Statistical analytical techniques and justification of techniques used: Results of the statistical analysis preferably in tabular form, methods for determining whether outliers exist, and justification for not using outliers.
- Ad hoc observations: These observations should include narrative descriptions of the study that do not fit into the previously described categories.
- **Discussion of the results**
- **Conclusions**

Appendices containing raw data, statistical results, and analytical data (EMSL, Libby, MT), etc. will be included in the final report. The Final Report of the study will be sent to the Sponsor after all comments on the draft report have been addressed and approved.

11. STUDY AMENDMENTS AND DEVIATIONS

Permanent changes to the final Study Protocol will require written amendments be prepared and submitted to the QAU Manager and Study Sponsor for approval. Any amendments will be reviewed to determine the potential impact on the study. If accepted, the amendment will be attached to the Study Protocol and become an active component of the study. Any deviations from the protocol (temporary changes due to unforeseen problems) will be recorded in the study records, dated, and initialed by the Study Director. Deviations will also be addressed in detail in the Final Report of the study.

12. RECORD MAINTENANCE AND ARCHIVAL

FEL will retain facility-related records (personnel training records, equipment calibration and maintenance records, storage temperature records, etc.) (13). No records will be disposed of without the authorization of USEPA and the Sponsor. The records will be organized and include an index.

Raw data, derived data, QA reports, study guidance documents, correspondence, and draft and final reports will be electronically maintained at the FEL facility in accordance with facility SOPs (13) until study finalization. Printed copies of the original study materials and report will be kept in designated file cabinets located in a secured file room at FEL. After final approval of all reports and the study has been officially concluded, all electronic files will be transferred to compact discs (CDs). All original CDs and original printed files associated with the study will be shipped to Golder Associates and archived. Storage of copies of all printed and electronic files will be maintained in a two-tier manner in accordance with FEL SOPs (14). Archives of copied files will first be kept in secure file cabinets, located in the study facility laboratory, for a period of one year or until the final report has been reviewed and accepted by

the client. After this time, the files will be transferred to storage file boxes and archived off premises at a secured commercial storage facility.

13. SPECIMENS ARCHIVAL

The preserved test specimens will be labeled and stored at FEL until study finalization in accordance with facility SOPs (15). Following study finalization, disposition of the study specimens will be determined by the Sponsor and FEL in consideration of potential future use (histopathology and endocrinology).

14. TEST SUBSTANCE WASTE DISPOSAL

Disposal of waste material generated by the study will be performed in accordance with those requirements provided in the facility SOPs (16). The sediment will be returned to the study site (Libby, MT) in accordance with those requirements provided in the facility SOPs. All wastewater associated with the conduct of the test will be collected and pumped through a filtering apparatus fitted with a 30 µm filter and 0.2 µm filter. The filters will then be shipped to the study site (Libby, MT) for disposal. Solid waste associated with the test will be placed in a designated trash receptacle and disposed according to federal, state, and local regulations.

15. QUALITY ASSURANCE UNIT (QAU)

The QAU is established as an objective, independent monitor of the work performed at FEL. The QAU will review data summaries prior to inclusion into the final report as part of the final report audit. Outside facilities providing support of the study will be responsible for auditing their data and reports through their QAU. The specific responsibilities for QA representatives from FEL include but are not limited to:

- Interact with the Study Director to ensure that personnel understand QA and Quality control (QC) procedures,
- Consult with the Study Director on actions required to correct deficiencies noted during the conduct of the in-life portion of the study,
- Ensure that all staff participating in the in-life portion of the study are adequately trained,
- Maintain complete facility and study-specific QA records related to the in-life portion of the study, and
- Review data reports and respective audits from support facilities.

The draft and final reports will be reviewed for protocol compliance, as well as to assure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the Study Director and management.

16. REFERENCES

1. USEPA, Sampling and Analysis Plan Operable Unit 3, Libby Asbestos Superfund Site, Phase V, Part B: 2012 Ecological Investigations, Revision 0, Denver, CO, April 2012.

2. FEL Quality Assurance Management Plan (QAMP), Fort Environmental Laboratories, Stillwater, OK, July 23, 2010 (current version).
3. Gosner. KL, A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. *Herpetologica* 16:183-191, 1960.
4. Fort, DJ, Degitz, S., Tietge J., and Touart. LW, The Hypothalamic-Pituitary Thyroid (HPT) Axis in Frogs and its Role in Frog Development and Reproduction, *Crit. Rev. Toxicol.* 37:117-161, 2007.
5. SOP 8.2, Adult frog care and maintenance, Fort Environmental Laboratories, 2000.
6. ASTM, Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, ASTM E 729-88, 1989.
7. SOP 7.10, Tissue homogenization and blood collection, Fort Environmental Laboratories, 2007.
8. OECD. Guidance Document on Amphibian Thyroid Histology. Environmental Health and Safety Publications. Series on Testing and Assessment, No. 82, Paris, France, 2007.
9. Organization of Economic Co-operation and Development (OECD). Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. Environ Health Safety Pub, Paris, 2006.
10. USEPA, Draft Guideline for the Testing of Chemicals. The Larval Amphibian Growth and Development Assay (LAGDA). November 2, 2010.
11. SOP 6.1.2, Sample Tracking and Handling, Fort Environmental Laboratories, 2010.
12. SOP 6.4, Receipt, Storage, and Distribution of Test Substances, Fort Environmental Laboratories, 2010.
13. SOP 4.10, Study File Document Collection and Organization, Fort Environmental Laboratories, 2001.
14. SOP 4.11, Maintenance of Study Archives, Fort Environmental Laboratories, 2001.
15. SOP 12.1, Archiving of Test Specimens, Fort Environmental Laboratories, 2003.
16. SOP 6.2, Waste Collection, Storage, and Disposal, Fort Environmental Laboratories, 2001.

APPENDIX A.2
Protocol for the Amphibian Field Collection Study
(Revision 0 – April 20, 2012)

APPENDIX A.2

Protocol for the Amphibian Field Collection Study

(Revision 0 – April 20, 2012)

1.0 Introduction

This protocol describes the standard operating procedures (SOPs) and sampling methods to be used when collecting and processing amphibians (various life stages) at Operable Unit 3 (OU3) and reference area collection sites.

2.0 Health and Safety

All field personnel engaged in the conduct of these *in situ* trout toxicity studies must follow health and safety protocols described in their firm's health and safety plan (and that reflect the nature of the specific work being accomplished). Asbestos fibers are easily inhaled when disturbed and when embedded in the lung tissue can cause health problems. Significant exposure to asbestos increases the risk of lung cancer, mesothelioma, asbestosis (non-cancerous lung disease), and other respiratory diseases (ATSDR 2006). Accordingly, in addition to other health and safety procedures established in a firm's health and safety plan, all field personnel working on OU3 will wear protective garments and respiratory protection appropriate to HAZWOPER protection Level C. Respiratory protection will be via Positive Air Pressure Respirators (PAPRs).

3.0 Background

Historic mining and milling operations at Operable Unit 3 (OU3) have resulted in the release of Libby Amphibole (LA) to the environment, including surface water and sediment in creeks and ponds in the Rainy Creek watershed. As part of the Remedial Investigation at OU3, the United States Environmental Protection Agency (EPA) is pursuing multiple lines of evidence to evaluate if exposure of aquatic life (benthic organisms, fish, amphibians) to Libby Amphibole (LA) in site surface water and sediment presents a potentially unacceptable risk.

No data exist on the effects of asbestos (LA) on developing amphibians. EPA will therefore consider two lines of evidence for evaluating risk to amphibians from LA: evidence (if any) from field collected amphibian life stages of any abnormal development or lesions that may have relevance to LA exposure in OU3 ponds, and conduct of a toxicity bioassay on the metamorphosis of larval amphibians (genus *Ranidae*) using LA-containing sediment collected from OU3. These lines of evidence were agreed upon by the OU3 Biological Technical Advisory Group (BTAG).

This protocol is focused on the specific methods and procedural details regarding the field collection of specific amphibian life stages from OU3 and reference ponds. The chief advantage of this line of evidence is that the exposure medium (sediment and surface water) will reflect actual site exposure conditions. A disadvantage is that it is not possible to control LA concentration levels so it is unlikely that a dose-response relationship can be established.

4.0 Definitions

Field Stage: 8 developmental windows representing a range of Gosner (1960) developmental stages

LA: Libby amphibole

NBF: Neutral buffered formalin

5.0 Responsibilities

This section presents a brief definition of field roles and the responsibilities generally associated with them. This list is not intended to be comprehensive and often additional personnel may be involved. Project team member information shall be included in project-specific plans (e.g., Sampling and Analysis Plan, etc.), and field personnel shall always consult the appropriate documents to determine project-specific roles and responsibilities. In addition, one person may serve in more than one role on any given project.

Project Manager: Selects site-specific sampling methods, sample locations, and constituents to be analyzed with input from other key project staff.

Quality Control Manager: Overall management and responsibility for quality assurance and quality control (QA/QC). Selects QA/QC procedures for the sampling and analytical methods, performs project audits and ensures that data quality objectives are fulfilled.

Field QA Officer: Performs audits of field activities being performed against procedures established in project SOPs for field work activities.

Field Team Leader (FTL): Implements the sampling program, supervises other sampling personnel. Also ensures compliance with SOPs and QA/QC requirements. Prepares daily logs of field activities.

Field Technicians (or other designated personnel): Assist the FTL in the implementation of field tasks. Perform the actual study activities, with the FTL, including study implementation (study set up, monitoring, sample collection, packaging, and documentation (e.g., sample label and log sheet, chain-of-custody record, etc)).

6.0 Amphibian Field Collection Study Procedures

The remainder of this section contains a detailed summary of the equipment and procedures for conducting the field collection of amphibian life stages. This study will collect various field stages of amphibians based on the anuran developmental stages originally identified by Gosner (1960).

6.1 Equipment

The following is a list of equipment that will be needed to conduct the amphibian field collection study.

- Global Position System (GPS) unit
- Flagging tape
- 66 surface water sample bottles (500-mL capacity, high-density polyethylene [HDPE] wide-mouth bottles to be provided by EMSL Analytical Inc. [EMSL] located in Libby)
- 26 sediment sample bottles (500 mL capacity HDPE wide mouth bottles (provided by EMSL) (Number includes sediment samples (candidate, OU3) for LA, organic and inorganic constituent as discussed for reference site selection, as well as routine sediment samples collected for LA analysis during the study)
- 52 Sediment sample labels (provided by EPA) (includes QA/QC samples and enough labels to affix to FSDS)
- 132 surface water sample labels (provided by EPA) (includes QA/QC samples and enough labels to affix to FSDS)
- 7-8 HOBO® TidbiT® submersible digital temperature loggers
- Laminated guide to amphibian field stages
- 8 large Tupperware containers with lids
- Gladware® containers
- Medium sized Tupperware® containers with lids
- 2 large rubbermaid bins/totes (with lid)

- Box sandwich-sized zip-top baggies
- Box gallon large zip-top baggies
- Magnifying glass (for field use)
- Large dipnets (several)
- Decontaminated stainless steel trowels (to be provided by Mike Chapman)
- Decontaminated stainless steel bowls (to be provided by Mike Chapman)
- Alconox soap (to be provided by Mike Chapman)
- Store bought distilled water
- Laboratory notebook (for offsite laboratory processing)
- Field logbook (for field work)
- Digital camera
- MS-222 (anesthetizing agent to euthanize amphibians)
- Disposable neoprene gloves
- Disposable scalpels
- Magnifying light
- Sharps container
- Roll of wax paper
- Top loading scale (400 g, 0.1 g precision)
- Top loading scale (100 g 0.001 g precision)
- Scale weights for daily calibration
- 2 sallons of store bought “spring” water
- Plastic sheeting
- Paper towels
- Sharpie brand markers (black)
- Ink pens
- Small, medium NBF pre-filled plastic jars with lids
- 1 large bottle of NBF to top off fill jars
- Sample labels (adhesive)
- OU3 Chain of Custody Forms (carbon copy triplicate;
- Sediment Sampling/Shipping Procedures for Sediment (**Attachment A**)
- Sediment Analytes for Candidate Reference Site Selection Activity (**Attachment B**)
- Approved Scientific Collection Permit (**Attachment C**)
- Pond Monitoring Data Sheet (**Attachment D**)
- Pond Tally Sheet (**Attachment F**)
- Rolls of clear packing tape
- 30 OU3 Phase V Part B Field Sample Data Sheets (FSDS) for surface water and sediment (**Attachment E**)
- Adhesive labels
- Coolers
- Strapping tape
- Custody seals

6.2 Study Design

The data needed for this line of evidence includes measurement of the nature and frequency of developmental abnormalities and lesions (if any) in amphibians collected from on-site (OU3) area ponds compared with that for amphibians of the same species collected from one or more reference ponds.

In addition, data are needed on the concentration of LA in water and sediment at the pond locations (Section 6.3) selected for evaluation. These data are not expected to be sufficient for developing an exposure-response relationship or for predicting effects in areas that are not studied, but are important to document that the areas selected for evaluation are contaminated with LA.

Three species will be targeted for capture in the reference and OU3 ponds, to the extent available in the field:

- Western Toad (*Bufo boreas*)
- Northern Tree Frog (*Pseudacris regilla*)
- Columbia Spotted Frog (*Rana luteiventris*)

For each of these species, egg masses and three (3) field stages will be sampled from each of the identified OU3 and reference ponds:

- Egg masses
- Field Stage 1: Premetamorphosis --- embryo-larval (Gosner stages 21-25)
- Field Stage 4: Prometamorphosis --- hindlimb development complete (Gosner stages 37-40)
- Field Stage 8: Metamorphic completion --- post climax (Gosner state 46)

6.3 Amphibian Collection Ponds

Here the ponds that will be targeted in the amphibian study are discussed.

Operable Unit 3:

The following four (4) ponds on OU3 are targeted for amphibian collections:

1. Tailings Pond. This is a large, 5-7 acre (estimated) pond characterized by surrounding marsh vegetation (cattails, etc).
2. Mill Pond. This is a smaller pond, approximately ½ acre, with similar habitat to the Tailings Pond.
3. Carney Pond. This is an approximately ¼ acre small creek-fed pond characterized by similar marsh habitat as the Tailings and Mill Ponds.
4. Fleetwood Pond. Another small creek-fed pond, approximately 1/8 acre with similar marsh habitat as Carney Pond.

Reference Ponds:

Several reference ponds are under consideration to support the amphibian field collection study. These reference ponds will be visited and sediment collected to determine the presence of chemicals and their concentrations, including: asbestos, metals, organic compounds; which could alter their use as suitable reference areas for the amphibian field study.

A total of six (6) candidate reference ponds will be investigated as potential reference ponds from among the following (only 3 ponds will be selected):

1. Bobtail Ponds. There are two (2) ponds here on private property above Bobtail Tributary. Permission has been obtained to use both ponds on this property (both are right next to each other). The ponds are both small (1/2 and 1/3 acre respectively) and would be similar in size and general habitat to Carney, Fleetwood and Mill Pond (which are respectively, approximately 1/4, 1/8 and 1/2 acres each). *If selected, these two ponds will be considered a single sampling unit (one pond) since they are right next to each other.*
2. Happy's Inn Ponds. There are several ponds in the Happy's Inn area, which is approximately 28 miles (as the crow flies) south east of OU3. All of the Happy's Inn ponds have habitat that is generally similar to the ponds at OU3 (marshy, wetland, shallow). Ponds/lakes in the Happy's Inn area that are under consideration include:
 - Banana Lake. A small pond/lake that is similar to Carney Pond

- Schneider Pond. A large pond that would be more similar to the Tailings Pond
- 3. National Heritage Site Ponds. There are many of these types of ponds. There are two (2) in particular under consideration: the Tepe ponds. These ponds are reasonably close to Libby. They are small ponds similar to Carney and Fleetwood Ponds. These ponds are currently snow covered and habitat conditions and similarity to OU3 ponds is unknown.

Chemistry Data to Support Reference Pond Selection

Sediments in the candidate reference ponds above will be characterized for chemistry and LA concentrations through the collection of a single composite sediment sample, comprised of 16 subsamples, from around each pond. These sediments will be collected in accordance with the procedures in **Attachment A**.

Sediment samples will be analyzed for LA and for a suite of non-asbestos constituents:

- Libby Amphibole (LA)
- PCBs (Aroclors)
- Organochlorine Pesticides
- Chlorinated Herbicides
- Metals
- Acid Volatile Sulfides
- Semi-volatile Organics
- Petroleum hydrocarbons
- Total organic carbon
- Moisture

EMSL Analytical (Libby, Montana) will perform the sediment LA analyses using the Libby-specific polarized light microscopy visual area estimation (PLM-VE) method.

Energy Laboratory in Billings, Montana, will perform all other sediment analyses.

Attachment B to this protocol is a table summarizing the analytical methods and reporting limits for the non-asbestos constituents listed above.

Though sediment data exists for OU3 ponds, for this study it is desirable to also have the same non-asbestos constituents listed above for reference pond selection be analyzed in a single composite sediment sample (also comprised of 16 subsamples). This provides OU3 sediment data for comparability with reference sediment in the event that abnormalities are identified. These composite samples will be collected once and temporally concurrent to the sediment samples collected to identify reference ponds. These OU3 and reference pond sediment samples collected during the reference site selection activity are wholly separate from the “routine” sediment samples collected following initiation of the amphibian study (i.e., at the early and late stages of organism development) and analyzed for LA concentrations (only). Collection methods are the same (**Attachment A**) for both sediment sample types (though only EMSL analyzes routine sediment samples).

Selection of the final reference ponds to be used in the amphibian field study will be based on the ponds with the lowest non-asbestos contaminant concentrations relative to each other, absence of LA, similarity of habitat to OU3 (all except Tepe Ponds, which are unknown, do have similar habitat), and ease of field staff access. If, during the conduct of the study, it is difficult to collect adequate specimens at the three selected ponds, a fourth pond from the candidate reference ponds may be considered.

6.4 Amphibian Study Sample Collection Goals

A scientific collection permit for amphibian (and trout) collection has been approved by the Montana Fish Wildlife and Parks (MFWP) (**Attachment C**). This permit will be physically in the possession of the field staff at all times. The goal of the collection program is to collect targeted number of organisms at various field stages.

The following numbers will be considered the amphibian sample goals for the study (fewer are likely at each location):

- Egg masses: 4 per species, per site (n=84)
- Field Stage 1 (premetamorphs): 40 per species (if available), per site (n=840)
- Field Stage 4 (prometamorphs): 40 per species (if available), per site (n=840)
- Field Stage 8 (metamorphs): 20 per species (if available), per site (n=420)

The actual number of organisms that will be collected is unknown, since species availability will vary at each location. It is not considered likely that each species will be available at each site or that all sampling goals will be met (species or numbers). It is anticipated that field sampling efforts to collect specimens at each field stage will range over the period spanning May through July or August. The actual collection frequency intervals will depend upon field conditions (and may be more frequent if development varies significantly across all sites).

Pond sites will be visited at least twice weekly to evaluate presence of amphibians, developmental stages and species. When present, developmental stages and species of interest will be collected.

Routine sediment and surface water samples will also be collected over the course of the study at each pond site to establish exposure conditions at the beginning and end of the developmental cycle.

Sediment and surface water sample requirements (i.e., "routine" samples) for LA analysis are as follows:

- OU3 Pond Surface Water:
 - One (1) sample weekly at each pond
- OU3 Pond Sediment:
 - One (1) sample at beginning of development (egg mass stage),
 - One (1) sample near end of development (Field stage 8)
- Reference Pond Surface Water:
 - One (1) sample at beginning of development (egg mass stage),
 - One (1) sample near end of development (Field stage 8)
- Reference Pond Sediment:
 - One (1) sample at beginning of development (egg mass stage),
 - One (1) sample near end of development (Field stage 8)

6.5 Pond Monitoring and Sampling Procedures

Monitoring

The exact time that amphibians breed and their eggs begin development depends on many environmental factors, especially temperature (air, water). Because of this, the timing of the study cannot be specified with certainty, so ponds will be monitored for water temperature and signs of amphibian breeding (egg masses). During twice weekly pond visits pond water temperature will be collected using a laser temperature reading device since water temperature is a key environmental cue for amphibian breeding. Ambient air temperatures (OU3 only) will be assessed remotely from the Mesowest meteorological station located on OU3 (accessible via the internet).

As noted above, field staff will check each pond site (OU3, reference) twice weekly starting in late April – early May) for signs of breeding (presence of egg masses). When egg masses begin to appear, sampling of the egg mass stage will begin (sampling continues until goals are met or until it is clear that goals will not be met easily). Pond monitoring activities, including collections of available organisms by developmental stage, will be recorded on an Amphibian Pond Monitoring Data Sheet (**Attachment D**). One sheet will be filled out for each pond and for each date that the pond is visited for monitoring and this data sheet will document amphibian and routine sediment/surface water sample collections. Notations on collections should also be made in the Field Logbook and on the FSDS (**Attachment E**) (i.e., the latter form is to document field-collected sediment and surface water samples only).

As the season progresses, opportunistic amphibian samples at desired developmental stages should be collected whenever a site is monitored to ensure that sampling goals are met as much as is reasonably possible.

Sediment Sampling

Sampling of sediments routinely for LA during the course of the amphibian study follows the collection and compositing procedures identified previously for reference site selection (see **Attachment A**). In brief, the sediment sample is based on a composite taken from 16 points around each pond (4 per “side”). A stainless steel trowel will be used and the sediments collected around the pond and placed in a sampling bottle provided by the laboratory. The sediments will be well mixed and debris (rocks, sticks, vegetation) removed. Water will be poured out and the sediment composite placed in a wide-mouth 500 ml HDPE bottle labeled with a pre-printed (supplied by EPA) sediment sample label (with pre-assigned sample number). The sample label is affixed to the sample jar using clear packing tape and an identical sample label is affixed to an FSDS (**Attachment E**). Collection of pond sediment samples during pond monitoring is recorded on the Pond Monitoring Data Sheet (**Attachment D**). Number and type of samples collected are also noted in the Field Logbook. The labeled bottle will be placed in a cooler for delivery to the Sample Preparation Facility (SPF) in Troy, MT:

EPA Sample Preparation Facility
303 N. 3rd Street
Troy, MT 59935
Project Contact: Andrea Wandler (406.295.9151 or awandler@techlawinc.com)

After preparation, the samples will be sent to EMSL for analysis:

EMSL Analytical, Inc.
107 West 4th Street
Libby, MT 59923
Project Contact: Ron Mahoney (406.293.9066 or Rmahoney@EMSL.com)

Surface Water Sampling

Routine sampling of surface water uses 500 mL HDPE wide mouth sample bottles pre-marked at the approximately 400 mL level. Bottles are not filled beyond this mark to permit headspace for later sonication/ozonation/UV irradiation by the analytical laboratory.

Surface water will be collected by dipping the lidded bottle beneath the pond surface and removing the lid to fill the bottle with no more than 400 mL of water, being careful not to disturb bottom sediments. The lid will be placed back on the bottle and the bottle will be wiped off and a pre-printed sample label affixed (EPA provides) that has a pre-assigned sample number. The label will be affixed using clear packing tape to the bottle. An identical label is affixed to the FSDS. Collection of surface water samples during pond monitoring is recorded on the Pond Monitoring Data Sheet (**Attachment D**), FSDS (**Attachment E**) and in the Field Logbook. The labeled bottles will be placed in a cooler for delivery to EMSL (see above for address and project contact).

Egg Mass Stage Sampling

Egg masses may be found in the water or (for spotted frogs) on vegetation in the water. To collect an egg mass:

- Put on a clean pair of sampling gloves (change at each pond)
- Gently remove the egg mass from the vegetation or just pick it up from the pond sediment and place in a labeled (using Duct Tape) Gladware container that contains some site water (multiple egg masses can be transported to the off-site laboratory in this manner)
 - Use magnifying glass and identify species using field identification guide
 - Place each species of egg mass in a different, labeled Gladware container
 - Using a piece of duct tape on Gladware container lid mark container with sampler name, pond name, species and date.
- Fill in this information on the Pond Monitoring Data Sheet (**Attachment D**) and make an entry in the Field Logbook
- Take representative photos and log frame numbers and descriptions in the Field Logbook (this need be done only occasionally; not for each collection event)
- Place the sealed, labeled Gladware containers in a cooler for transport back to the off-site laboratory for processing.

Tadpole Sampling (Field Stages 1 and 4)

Tadpoles in the water will generally be located in the shallows near pond edges. Use of chest waders is recommended to ensure that the sampler is kept dry during organism collection. Collection occurs as follows:

- Put on a fresh pair of sample gloves at each pond where collection will occur
- Using a dipnet, collect a number of tadpoles and put them in a large Gladware container filled with some pond water
- Individually stage and identify species in your container using the field guide and magnifying glass
- Place each species in a separate, labeled Gladware container with some site water
 - On a piece of duct tape affixed to the Gladware lid, record the pond name, species, field stage date and sampler name.
- Fill in this information on the Pond Monitoring Data Sheet (**Attachment D**) and make an entry in the Field Logbook
- Release any tadpoles that are not of the desired field stage or that are not a permitted species for collection
- Repeat this until the desired number of tadpoles is obtained at each pond for each species and field stage
- Place labeled water filled Gladware containers into a large cooler for transport to the off-site laboratory for processing.

Metamorphosed Juvenile Sampling (Field Stage 8)

Young, metamorphosed juveniles will be located in or near the water's edge (not always in the water but near). Use of chest waders is recommended to ensure that the samplers are kept dry during organism collection. Collection will occur as follows:

- Put on a fresh pair of sample gloves at each pond where collection will occur
- When a young frog is seen, gently scoop the frog with the dipnet and place in a Gladware container
- Identify species and stage the frog using the field guide and magnifying glass
 - It is acceptable to collect metamorphs with a small tail bud remaining

- Place each species in a separate labeled Gladware container with a small amount of site water (for transport to off-site laboratory for processing)
 - On a piece of duct tape on the lid of the container label with pond name, species, field stage, sampler name, date
- Fill in this information on the Pond Monitoring Data Sheet (**Attachment D**) and make an entry in the Field Logbook
- Release any organisms that are not of the desired developmental stage or that are not a permitted species for collection
- Repeat this until the desired number of species field stages are obtained
- Place labeled Gladware containers into a large cooler for transport to the off-site laboratory for processing.

6.6 Processing Amphibians in the Off-site Laboratory

Given a number of factors including the high potential number of organisms that could be collected, the extreme number of sample containers that would be required if individual organisms are preserved individually and the fact that fresh weights do not differ significantly between fresh and preserved organisms (according to Dr. Fort who routinely does this), individual organism sample weights and measures will not be collected for amphibians at the off-site laboratory. Instead, weights and measures for each organism will be provided by Fort Environmental Laboratory. Weights and lengths are recorded along with other observations during the external exam conducted by Fort Environmental Laboratory for each amphibian,

The assigned sample numbers by Fort Environmental Laboratory will be easily traceable back to the sample container identification number assigned in the off-site processing lab (i.e., reflecting the original pond, collection date, species, developmental stage and number of organisms). Specimen sample container identification numbers for specimen containers sent to Fort Environmental Laboratory will be assigned as discussed in Section 6.7.

Euthanasia and Preservation of Egg and Tadpole Groups (Field Stages 1 and 4 Only)

Groups of organisms of like developmental stage, collection pond, species and collection date are euthanized and preserved together in an NBF-filled jar as follows:

- Clean neoprene gloves
- In a large size Gladware container place one liter of “store bought” spring water
- Weight out 250 mg of MS-222 on a piece of wax paper
- Pour MS-222 into water and cover with lid
- Gently stir and/or shake from side to side until fully dissolved
- Remove lid
 - Note: keep MS-222 mixture covered with lid when not in use
 - A fresh batch of MS-222 is mixed when a batch is no longer effective in euthanizing
- Place “like” organisms (i.e., those that will be sent in a single NBF-filled jar) in small batches in MS-222 solution until no longer moving (ok if breathing though)
- Place all anesthetized animals whole (no incisions) in a pre-filled (NBF), lidded container for preservation
- Label the NBF container with the specimen container identification number following the procedures in Section 6.7.
- Fill in a Pond Sample Tally Sheet (**Attachment F**). This sheet will be continually filled in for a given pond until the samples goals are achieved by species and developmental stage, at which time the Tally Sheet will be complete (or as complete as is possible)
- Place sealed, labeled NBF-filled container with pond specimens in a ziplock bag and place in cooler (to go to Fort Environmental Laboratory)

- All life stages sent to Fort Environmental Laboratory will have weights, lengths and external examinations
 - Fort Environmental Laboratory is responsible for performing organism necropsy and sending field stage 8 specimens to Northwest ZooPath for histology
- Repeat euthanizing and preservation procedure for all groups of egg masses and tadpoles

Euthanasia and Preservation of Metamorph Groups (Field Stage 8 Only)

- Place organisms in small batches in MS-222 solution until no longer breathing (no movement of animal's sides)
- Remove organisms one at a time and open the body cavity of the organism for proper preservation as follows:
 - Using lighted magnifying lamp and a clean disposable scalpel:
 - Make shallow incision on ventral side from beneath mouth to anus
 - CAUTION: skin is thin on these animals. So incisions should be done gently and shallowly to ensure only the external skin and internal peritoneal lining is incised (do not cut through organs)
 - Blot off blood
- Place "opened" animals from the same group into a pre-filled (NBF), lidded container and close lid very tightly
- Label the NBF container with the specimen container identification number following the procedures in Section 6.7.
- Place NBF filled container with organisms in a ziplock bag and place in a cooler (to go to Fort Environmental Laboratory)
 - All life stages sent to Fort Environmental Laboratory will have weights, length measures and external examinations
 - Fort Environmental Laboratory is responsible for performing organism necropsy and sending metamorphs on to Northwest ZooPath for histology
- Repeat euthanizing and preservation procedure in this manner for all groups of metamorphs
- Dispose of scalpel(s) in sharps container.

6.7 Assigning Specimen Container Identification Numbers

Sample container identification numbers are assigned to a given group of amphibians of the same species, developmental stage, pond and collection date. The containers are coded with the following information:

Pond Name: Two initials BT (Bobtail); BL (Banana Lake) SP (Schneider Pond), TP (Tailings Pond), MP (Mill Pond), TPE (Tepe pond), FP (Fleetwood Pond), CP (Carney Pond) etc.

Developmental Stage:
 EG (egg mass)
 PRE (Field Stage 1)
 PRO (Field Stage 4)
 MM (Field Stage 8)

Species: TF (tree frog); SF (spotted frog); WT (western toad); O (other)

Date: Collection date (mm/dd/yy)

No. of Organisms: The total number of specimens in the sample container

Example Specimen Container Identification Numbers:

An NBF container with 11 spotted frog tadpoles, field stage 4, collected at the Tailings Pond on June 1, 2012, would have the following specimen container identification number:

TP-PRO-SF-060112-11

The 11 at the end of the number designates the number of organisms collected in that species and field stage, at that pond on that date.

An NBF container with 6 western toad metamorphs collected at Bobtail Ponds on July 17, 2012, would have the following specimen container identification number:

BP-MM-WT-071712-6

Each of the organisms in these groups will have external exams, weights and measures recorded by Fort Environmental Laboratory. Metamorph necropsy will be performed by Fort Environmental Laboratory and the organisms submitted to Northwest ZooPath for histology.

6.8 Assigning Sample Numbers to Surface Water and Sediment Samples

All surface water and sediment samples should be labeled using the pre-printed labels, with pre-assigned sample numbers provided by EPA (see Section 8.5.1 of the main SAP/QAPP for details). A label is affixed to each sediment or surface water sample container using clear packing tape and an identical label is also affixed to the FSDS (and also recorded in the Field Logbook).

6.9 External Examinations of Amphibians (Fort Environmental Laboratory)

Following off-site laboratory processing, all specimens will be shipped to Fort Environmental Laboratory where external developmental exams, including weights and measures, will be performed on all organisms collected, regardless of species or field stage. External development will be conducted on the following features, recorded and reported (Data Report, electronic database) for each developmental stage as follows:

1. Egg Mass Stage:
 - a. structure
 - b. cleavage
2. Pre- and Prometamorphosis (Larval) Stages:
 - a. Mouth
 - b. Gills
 - c. Eyes
 - d. Skin
 - e. Tail
 - f. Limbs
 - i. Hindlimb length (HLL)/snout-vent length (SVL) – photo-digitization
3. Metamorphosed young:
 - a. Mouth
 - b. Eyes
 - c. Skin
 - d. Limbs

External exams, organism weights and measures will be recorded on standard Fort Environmental Laboratory forms (electronic). Results of the external exams will result in the following data being generated:

- Total number of abnormal specimens by stage, species, and site
- Total of each specific abnormality by stage, species, and site
- Co-occurrence of abnormalities to determine potential syndromes by stage, species, and site
- Determination of severity weighting index as identified in Section 6.11 to determine an overall score that can be applied across sites.

Once all organisms have been examined and scored, Fort Environmental Laboratory will evaluate which of the three amphibian species represented has the most complete developmental data set (i.e., considering how many stages are represented and the number of samples collected for each stage) for each pond site (OU3 and reference). The field stage 8 animals from this developmental data set, for each pond, will be sent to Northwest ZooPath individually in separate containers, each with the original specimen container ID number and organism number (e.g., organism number such as 1 of 6 for example), as well as the Fort Environmental Laboratory sample identification number, for histology.

The Fort Environmental Laboratory data report will identify this complete data set for metamorphs for each site. Results of exams, necropsy (metamorphs only), and scoring will also be provided in an electronic database and statistical analysis completed as part of the Fort Environmental Laboratory data report. The Fort Environmental data report will be provided to Golder, who will include it as an appendix to a larger data report prepared for the study. The Golder draft data report, which will also include Northwest ZooPath's data report, will be submitted to EPA electronically upon completion. Golder will upload electronic databases provided by Fort Environmental Laboratory (and Northwest ZooPath) to the eRoom when provided to Golder.

6.10 Necropsy and Histology of Metamorphs

Metamorph necropsy will be conducted by Fort Environmental Laboratory. Northwest Zoopath (Dr. Michael Garner, board-certified veterinary pathologist) will receive the preserved (NBF) field stage 8 animals from Fort Environmental Laboratory for histology. Fort Environmental Laboratory will conduct necropsies using standard methods for each metamorph. Necropsy findings will be tabularized for each animal by sample container identification number and organism number using Fort Environmental Laboratory forms or spreadsheets.

Following necropsy, the animal will have the following tissues excised, embedded, sliced and stained (various stains may be used) by Northwest ZooPath:

- Mouth
- Skin
- Gonadal tissue

Gills were identified for histology in error since metamorphs are air breathers. The tissues identified above may have a potential to be affected by asbestos, though there is limited data in the scientific literature on the effects of asbestos on any aquatic animals including amphibians. Other major organ groups may also be examined histologically by Dr. Garner.

Sample numbers (both Golder and Fort Environmental Laboratory) on the specimen containers provided to ZooPath will be recorded for each organism and tissue prior to necropsy and reading (respectively) and these sample identification numbers referenced to all observations provided by the pathologist. Representative sections of each tissue will be selected and examined microscopically for any abnormalities or lesions. The presence and type of each lesion will be recorded for each animal's tissues.

The tissues will be tabulated and a score assigned to each tissue examined based on the following:

<u>Lesion Severity</u>	<u>Score Assigned</u>
No lesion	0
Minimal lesion	1
Mild lesion	2
Moderate lesion	3
Marked lesion	4
Severe lesion	5

In addition to severity and score, the frequency of observed lesions will also be recorded for each tissue evaluated. Representative microphotographs will be taken to illustrate what the pathologist identifies as meeting the lesion types identified above. These will be included in the report provided by Northwest ZooPath to Golder.

The lesion type will be multiplied by a pathos factor of either 1 or 2, recorded by the pathologist, to address if the lesion is believed to be potentially attributable to asbestos (factor of 2) or other (non-asbestos) causes (factor of 1). Lesions that occur naturally in reference (control) animals will be useful in evaluating and considering attribution for animals collected from the asbestos impacted ponds. If the observed lesion is considered to be of natural origin (i.e., parasite, disease) the lesion score will be multiplied by a "pathos" factor of either 1 or 2. A Pathos factor of 1 indicates no attribution of the lesion is likely from asbestos fibers. A Pathos factor of 2 indicates a potential likelihood that the observed lesion or abnormality may be related to asbestos (and reasoning presented). The pathologist's report will clearly indicate the state of the animals health (necropsy) and whether any observed effects are, in the pathologist's judgment, potentially related to asbestos or to other unknown causes.

The report by Northwest ZooPath will be transmitted to Golder electronically and will be included in Golder's draft data report prepared for the amphibian study. Electronic deliverables from Northwest ZooPath will be uploaded to the eRoom when provided to Golder. Golder's draft report will be provided to EPA for comment.

6.11 Data Backup and Biweekly Data Submissions to EPA

Digital photos should be downloaded to a laptop as a secondary backup from the camera and then uploaded bi-weekly to the OU3 eRoom.

Also bi-weekly (or more frequently as conditions permit), pages from the Field Logbook, pond monitoring sheets, FSDSs and laboratory logbook pages should be scanned and uploaded to the OU3 eRoom. This is accomplished by going to the Grace office in Libby with a laptop, scanning the required pages using the office copy/scanner and then uploading the data to the following web address:

<https://team.cdm.com/eRoom/mt/LibbyOU3>. Individual OU3 eRoom access codes are assigned to Golder staff working on the project and these access codes will be used by individuals uploading data. Electronic deliverables from Fort Environmental Laboratory and Northwest ZooPath will be uploaded to the eRoom when provided to Golder.

Questions or problems with data uploading to the OU3 eRoom should be directed to Lynn Woodbury (CDM Smith): woodburyl@cdmsmith.com (303) 383-2382.

7.0 References Cited

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.

ATTACHMENT A:
LIBBY OU3: PHASE V SAP, PART B, REV 0
SEDIMENT SAMPLING / SHIPPING PROCEDURES:
REFERENCE POND SELECTION, CONCOMMITANT OU3 POND SAMPLING

1. Overview

Sampling of pond sediment is needed to provide a snapshot of concentrations of LA, organic and inorganic constituents in pond sediment for candidate reference ponds and OU3 ponds. Data for the candidate reference ponds is used to actually select the reference ponds to be monitored in the study. Data for OU3 ponds is collected to provide a concordant (temporal and chemical) data set (i.e., with that of the selected reference ponds) that can be later used in interpreting field amphibian development data in the event abnormalities should be identified.

Sediment sampling equipment, locations, and procedures are identified in this document. Sediment sampling and decontamination procedures identified are consistent with those contained in the OU3-specific Standard Operating Procedure (SOP) No. 5, *Sediment Sampling* (see Appendix B of the Phase V Part B SAP/QAPP).

Ten (10) ponds will have sediment sampled. Four (4) of these ponds are from OU3 and six (6) of the ponds represent “candidate” reference ponds¹. A composite sediment sample is collected from the top 2-inches of sediment, just inside the water line, around all sides of each pond (candidate reference, OU3). These sediments are placed in a stainless steel bowl, mixed and then divided among sampling containers to be sent to the analytical laboratories (EMSL Analytical [EMSL], Energy Laboratory [Energy]) for various analyses. Stainless steel sampling bowls and trowels must be decontaminated prior to first use and prior to any re-use at another pond site.

The remainder of this document describes the equipment and procedures for collecting, labeling, storing and shipping sediment samples collected from candidate and selected reference ponds, and OU3 ponds.

2. Equipment.

- Stainless steel mixing bowls
- Stainless steel trowels
- Disposable neoprene gloves
- Alconox soap
- Distilled water
- Ice punch (if needed)
- 10 500-mL wide mouth HDPE plastic bottles with cap/lid (provided by EMSL)
- 40 (plus extra for breakage) 4-ounce jars with lids (for Energy Lab samples)
- Paper towels
- Trash bag (for used paper towels, gloves)
- Pre-printed labels with pre-assigned sample numbers (assigned by EPA)
- Field Sample Data Sheets (FSDS)
- Permanent marking pen (Sharpie)
- Carbon triplicate Chain Of Custody forms
- Ink pen (for filling in Chain of Custody forms)
- Large zip-top baggie (for Chain of Custody form to Energy)
- Strapping tape

¹ Selection of amphibian reference sites will be made after sediment analytical results are received and reviewed, likely by late April or early May.

- Custody seals
- Blue ice (to keep sediment samples cool during collection and shipment)
- Bubble wrap or other packing material (to minimize breakage)
- Cooler(s) (for shipping sample bottles to Energy Labs in Billings, MT)

3. Candidate Reference Pond Selection: Sediment Sampling (Total of 6)

A total of six (6) candidate reference ponds will have sediment sampled. They are:

4. Bobtail Ponds. There are two (2) ponds here on private property (Barell Family; permission obtained to use), which is above Bobtail Tributary. Permission has been obtained to use both ponds on this property (both are right next to each other). The ponds are both small (1/2 and 1/3 acre respectively) and would be similar in size and general habitat to Carney, Fleetwood and Mill Pond (which are respectively, approximately 1/4, 1/8 and 1/2 acres each). If selected, these two ponds will be considered a single sampling unit (one pond) since they are right next to each other.
5. Happy's Inn Ponds. There are several ponds in the Happy's Inn area, which is approximately 28 miles (as the crow flies) south east of OU3. All of the Happy's Inn ponds have habitat that is generally similar to the ponds at OU3 (marshy, wetland, shallow). Ponds/lakes in the Happy's Inn area that will have sediments sampled are:
 - Banana Lake. A small pond/lake that is similar to Carney Pond
 - Schneider Pond. A large pond that would be more similar to the Tailings Pond
6. National Heritage Site Ponds. There are many of these types of ponds. There are two (2) in particular under consideration: the Tepe ponds. These ponds are reasonably close to Libby. They are small ponds similar to Carney and Fleetwood Ponds. These ponds are currently snow covered and habitat conditions and similarity to OU3 ponds is unknown.

4. OU3 Pond Sediment Sampling for LA, Organic and Inorganic Constituents (Total of 4)

Each pond on OU3 will have a sediment composite sample collected. These ponds are:

- Tailings Pond
- Mill Pond
- Carney Pond
- Fleetwood Pond

5. Equipment Decontamination Procedure

- Prior to first use, stainless steel bowls and trowels will be decontaminated as follows:
 - Wash sampling equipment (bowls, trowels) with distilled water and Alconox brand soap.
 - Rinse well with distilled water (at least 3 full rinses to remove soap).
 - Dry with paper towels.
 - Wrap decontaminated equipment in foil until use.
- Re-use of bowls or trowels at another pond site requires additional decontamination following the steps identified above.
- Paper towels are discarded in a trash bag.

6. Pond Sediment Sampling Procedure

- Put on a fresh pair of neoprene gloves.
- Using the trowel, remove the top two (2) inches of sediment from just inside the water line in a number of locations around each side of the pond:
 - Collect composite from 16 locations around each pond: 4 on each "side" of the pond. These subsamples should be relatively evenly spaced around the pond perimeter for a total of 16 subsamples.
 - Given current ice and snow conditions this may require an ice punch.
- Place all sediment subsamples in the clean stainless steel mixing bowl. Ensure plenty of sediment is collected in each location around the pond (there are many jars to fill for sample analysis).
- Thoroughly mix the sediments in the bowl using the trowel.
- Once thoroughly mixed, remove large debris such as ice chips, large rocks, sticks and vegetation.
- Pour off any water in the mixing bowl.
- Divide sediment among sampling containers for each analytical laboratory as follows:
 - EMSL Sample (for LA):
 - One 500-mL HDPE wide-mouth bottle. Fill the bottle. Wipe off the outside of the bottle with a paper towel after filled
 - Place a pre-printed sediment sample label (provided by EPA) on the bottle
 - Place an identical sample label on an FSDS
 - Place labeled bottle in cooler with blue ice to keep cool.
 - Energy Samples (full suite of metals, organics):
 - Four- 4-ounce glass sampling jars are used.
 - Each is filled to allow sufficient sediment for Energy to conduct the many analytical analyses requested. Make sure each of the bottles is completely full.
 - Wipe off bottles with paper towels
 - Place a pre-printed sediment sample label (provided by EPA) on each bottle (each of these four labels should have the same sample ID number since they are from the same pond)
 - Place one identical sample label on the FSDS
 - Place labeled sample bottles into a cooler with blue ice to keep cool.

7. Sediment Chemistry

All pond sediment samples (candidate reference, OU3 sites) will be analyzed for the following constituents (responsible laboratory identified):

- | | |
|-----------------------------|----------|
| ○ Libby Amphibole (LA) | (EMSL) |
| ○ Total PCBs | (Energy) |
| ○ Organochlorine Pesticides | (Energy) |
| ○ Chlorinated Herbicides | (Energy) |
| ○ Metals | (Energy) |
| ○ SVOCs | (Energy) |
| ○ Diesel range organics | (Energy) |
| ○ Gasoline range organics | (Energy) |
| ○ Total organic carbon | (Energy) |
| ○ Acid Volatile Sulfides | (Energy) |
| ○ Moisture | (Energy) |

Sediment samples will be analyzed for LA using the Libby-specific PLM-VE method. **Attachment B** identifies the applicable analytical methods and reporting limits for non-asbestos constituents.

8. Chain of Custody (COC) Form and Shipping

- EMSL
 - Fill out the COC identifying bottles by pond name and sample number (carbon triplicate form is used)
 - Specify sediment (or SD) in the media column.
 - Specify Libby Amphibole (LA) as the analyte.
 - The Libby-specific PLM-VE is the requested method.
 - Sign the form and hand-deliver the bottles with COC form to the Sample Preparation Facility (SPF) in Troy, MT:

EPA Sample Preparation Facility
 303 N. 3rd Street
 Troy, MT 59935
 Project Contact: Andrea Wandler (406.295.9151 or awandler@techlawinc.com)

- The SPF project contact will ensure that sediment samples are sent to EMSL in Libby for analysis following sample preparation.

- Energy
 - Fill out the COC identifying number of bottles by pond name (should be 4 bottles of sediment per pond).
 - Specify sediment (or SD) in the media column.
 - In the area identifying chemicals desired for analysis find a blank line and write in "see attached analyte list". This list is provided in **Attachment B** and a copy should be clipped to the COC form.
 - Sign the COC form.
 - Pack the cooler (see below) with blue ice and include COC form. If more than one cooler is used, a separate COC form is filled out to identify contents of second cooler.

9. Pack Cooler for Shipping to Energy

Procedures here are consistent with those in OU3 SOP No. 8 – *Sample Handling and Shipping* (see Appendix B of the Phase V Part B SAP/QAPP).

- Place each labeled sample bottle into plastic ziplock baggie (one bottle per bag) and seal to protect against breakage.
- Place all sample bottles in the cooler(s) with blue ice to keep samples cool during shipment to Energy Labs.
- Packing such as newspaper, bubble wrap, etc. can be placed in among bottles to reduce contact and breakage potential.
- Inventory cooler contents (number of bottles) and fill out COC form.
- Place COC and attached analyte list in a large plastic ziplock baggie and tape this bag to the inside of the lid of the cooler. If more than one cooler is used, follow the same procedure for the second cooler.
- Seal the cooler using strapping tape (wrap around the body and lid on both ends of the cooler multiple times).
- Add a custody seal.
- Telephone Energy (see contact information below) to confirm shipping address and to confirm that someone will be there to receive the samples.
- Send sealed cooler (next day delivery) to Energy, Billings, Montana :

Energy Laboratories
 1120 South 27th Street (59101)
 Billings, MT 59107-0916
 Phone: 1-800-735-4489

ATTACHMENT B
LIBBY OU3: PHASE V SAP, PART B, REV 0
SEDIMENT ANALYTES FOR CANDIDATE REFERENCE
SITE SELECTION ACTIVITY

Test Method	Analyte	Reporting Limit
SW 8081A	Organochlorine Pesticides	
	4,4'-DDD	0.0017 mg/kg
	4,4'-DDE	0.0017 mg/kg
	4,4'-DDT	0.0017 mg/kg
	Aldrin	0.0017 mg/kg
	alpha-BHC	0.0017 mg/kg
	alpha-Chlordane	0.0017 mg/kg
	beta-BHC	0.0017 mg/kg
	Chlordane	0.017 mg/kg
	delta-BHC	0.0017 mg/kg
	Dieldrin	0.0017 mg/kg
	Endosulfan I	0.0017 mg/kg
	Endosulfan II	0.0017 mg/kg
	Endosulfan sulfate	0.0017 mg/kg
	Endrin	0.0017 mg/kg
	Endrin aldehyde	0.0017 mg/kg
	Endrin ketone	0.0017 mg/kg
	gamma-BHC (Lindane)	0.0017 mg/kg
	gamma-Chlordane	0.0017 mg/kg
	Heptachlor	0.0017 mg/kg
	Heptachlor epoxide	0.0017 mg/kg
	Methoxychlor	0.0017 mg/kg
	Toxaphene	0.167 mg/kg
SW 8151A	Herbicides, Chlorinated	
	2,4,5-T	0.004 mg/kg
	2,4,5-TP (Silvex)	0.004 mg/kg
	2,4-D	0.02 mg/kg
	2,4-DB	0.05 mg/kg
	3,5-Dichlorobenzoic Acid	0.01 mg/kg
	4-Nitrophenol	0.01 mg/kg
	Acifluorfen	0.01 mg/kg
	Bentazon	0.05 mg/kg
	Chloramben	0.01 mg/kg
	Dacthal	0.02 mg/kg

	Dalapon	0.05 mg/kg
	Dicamba	0.005 mg/kg
	Dichlorprop	0.02 mg/kg
	Dinoseb	0.02 mg/kg
	MCPA	4 mg/kg
	MCPP	4 mg/kg
	Pentachlorophenol	0.002 mg/kg
	Picloram	0.01 mg/kg
ASA 29-3	Carbon, Total Organic	
	Inorganic Carbon by difference	0 wt%
	Organic Carbon	0 wt%
	Organic Carbon, Total (TOC)	0 wt%
	Organic Matter	0 wt%
	Organic Matter, Total (TOM)	0 wt%
SW 8015B	Diesel Range Organics	
	Diesel Range Organics (DRO)	10 mg/kg
	Total Extractable Hydrocarbons	10 mg/kg
SW 8015B	Gasoline Range Organics	
	Gasoline Range Organics (GRO)	2 mg/kg
	Total Purgeable Hydrocarbons	2 mg/kg
SW7471A	Mercury in Solid By CVAA	
	Mercury	1 mg/kg
E6010.20	Metals by ICP/ICPMS, Total or Soluble	
	Aluminum	5 mg/kg
	Antimony	1 mg/kg
	Arsenic	1 mg/kg
	Barium	1 mg/kg
	Beryllium	1 mg/kg
	Cadmium	1 mg/kg
	Chromium	1 mg/kg
	Cobalt	1 mg/kg
	Copper	1 mg/kg
	Iron	5 mg/kg
	Lead	1 mg/kg
	Magnesium	5 mg/kg
	Manganese	1 mg/kg

	Nickel	1 mg/kg
	Selenium	1 mg/kg
	Silver	1 mg/kg
	Strontium	1 mg/kg
	Tin	1 mg/kg
	Vanadium	1 mg/kg
	Zinc	1 mg/kg
D2974	Moisture	
	Moisture (As Received)	0.2 wt%
ASA 10-3	pH, 1:X Water Extractable	
	pH, 1:1	0.1 s.u.
SW 8082	Polychlorinated Biphenyls (PCB's)	
	Aroclor 1016	0.017 mg/kg
	Aroclor 1221	0.017 mg/kg
	Aroclor 1232	0.017 mg/kg
	Aroclor 1242	0.017 mg/kg
	Aroclor 1248	0.017 mg/kg
	Aroclor 1254	0.017 mg/kg
	Aroclor 1260	0.017 mg/kg
	Aroclor 1262	0.017 mg/kg
	Aroclor 1268	0.017 mg/kg
SW 8270C	Semi-Volatile Organic Compounds	
	1,2,4-Trichlorobenzene	0.333 mg/kg
	1,2-Dichlorobenzene	0.333 mg/kg
	1,3-Dichlorobenzene	0.333 mg/kg
	1,4-Dichlorobenzene	0.333 mg/kg
	1-Methylnaphthalene	0.333 mg/kg
	2,4,5-Trichlorophenol	0.333 mg/kg
	2,4,6-Trichlorophenol	0.333 mg/kg
	2,4-Dichlorophenol	0.333 mg/kg
	2,4-Dimethylphenol	0.333 mg/kg
	2,4-Dinitrophenol	1.67 mg/kg
	2,4-Dinitrotoluene	0.333 mg/kg
	2,6-Dinitrotoluene	0.333 mg/kg
	2-Chloronaphthalene	0.333 mg/kg
	2-Chlorophenol	0.333 mg/kg
	2-Methylnaphthalene	0.333 mg/kg

	2-Nitrophenol	0.333 mg/kg
	3,3'-Dichlorobenzidine	0.333 mg/kg
	4,6-Dinitro-2-methylphenol	1.67 mg/kg
	4-Bromophenyl phenyl ether	0.333 mg/kg
	4-Chloro-3-methylphenol	0.333 mg/kg
	4-Chlorophenol	0.333 mg/kg
	4-Chlorophenyl phenyl ether	0.333 mg/kg
	4-Nitrophenol	1.67 mg/kg
	Acenaphthene	0.333 mg/kg
	Acenaphthylene	0.333 mg/kg
	Anthracene	0.333 mg/kg
	Azobenzene	0.333 mg/kg
	Benzidine	0.333 mg/kg
	Benzo(a)anthracene	0.333 mg/kg
	Benzo(a)pyrene	0.333 mg/kg
	Benzo(b)fluoranthene	0.333 mg/kg
	Benzo(g,h,i)perylene	0.333 mg/kg
	Benzo(k)fluoranthene	0.333 mg/kg
	bis(-2-chloroethoxy)Methane	0.333 mg/kg
	bis(-2-chloroethyl)Ether	0.333 mg/kg
	bis(2-chloroisopropyl)Ether	0.333 mg/kg
	bis(2-ethylhexyl)Phthalate	0.333 mg/kg
	Butylbenzylphthalate	0.333 mg/kg
	Chrysene	0.333 mg/kg
	Di-n-butyl phthalate	0.333 mg/kg
	Di-n-octyl phthalate	0.333 mg/kg
	Dibenzo(a,h)anthracene	0.333 mg/kg
	Diethyl phthalate	0.333 mg/kg
	Dimethyl phthalate	0.333 mg/kg
	Fluoranthene	0.333 mg/kg
	Fluorene	0.333 mg/kg
	Hexachlorobenzene	0.333 mg/kg
	Hexachlorobutadiene	0.333 mg/kg
	Hexachlorocyclopentadiene	0.333 mg/kg
	Hexachloroethane	0.333 mg/kg
	Indeno(1,2,3-cd)pyrene	0.333 mg/kg
	Isophorone	0.333 mg/kg
	m+p-Cresols	0.333 mg/kg
	n-Nitroso-di-n-propylamine	0.333 mg/kg
	n-Nitrosodimethylamine	0.333 mg/kg
	n-Nitrosodiphenylamine	0.333 mg/kg

	Naphthalene	0.333 mg/kg
	Nitrobenzene	0.333 mg/kg
	o-Cresol	0.333 mg/kg
	Pentachlorophenol	1.67 mg/kg
	Phenanthrene	0.333 mg/kg
	Phenol	0.333 mg/kg
	Pyrene	0.333 mg/kg
	Pyridine	0.333 mg/kg
AVS/TTR	Sulfide, Acid Volatile	
	Acid Volatile Sulfide	20 mg/kg
ASA 33-7	Ammonia as N, KCL extract	1 mg/kg

ATTACHMENT C
LIBBY OU3: PHASE V SAP, PART B, REV 0
AMPHIBIAN COLLECTION PERMIT

**MONTANA FISH, WILDLIFE & PARKS
SCIENTIFIC COLLECTORS PERMIT
FISHERIES**

Permit Number:	02-2012	Date Issued:	4/2/2012
Fee Received:	Yes	Date Expires:	12/31/2012
Permit Issued to:	Robinson, Sue 18300 NE Union Hill Road Redmond WA 98052		
Associated With:	Golder Associates Inc., Environmental Consulting Firm		
Associates Name:	Jeremy Clark		

Permission is given to take, kill, capture, or possess, in accordance with the provisions of Section 87-2-806, MCA, the following:

Authorized to use electrofishing, block nets and modified minnow traps to collect up to 10 trout (representing any of the various species present) from each of the two size categories (<65 mm, >65 mm - < 100 mm) from both Upper and Lower Rainy Creeks on the Libby Asbestos Mine Site, and at each of the two off-site reference locations: Bob Tail Tributary and Noisy Creek. Fish shall be euthanized and may be retained for developmental examinations and possible histopathological evaluation. Therefore, a maximum of 40 fish (mix of species) may be collected from the Mine Site (OU3), and a maximum of 40 fish collected from the two reference locations (maximum sample sizes are inclusive of two size categories). Sampling may begin on August 15, 2012 and continue until all fish have been collected. All fish larger than 100 mm must be returned live to the stream near the point of capture. In addition, up to 10 adult trout (of any size) may also be collected from the OU3 on-site Tailings Pond and submitted for analysis of asbestos to support the human health risk assessment being conducted by USEPA Region 8.

Dipnets may also be used to collect amphibians (western toads, Columbia spotted frogs, northern tree frogs) at multiple stages of their development ranging from eggs to metamorphosed juveniles. These organisms may be collected at four ponds (Fleetwood, Carney, Tailings, and Mill Ponds) located on the Libby Mine Site (OU3), and from 3 reference ponds including Bob Tail ponds on private property, and two other ponds yet to be identified. Animals must be euthanized and may be submitted for examinations and histology to assess potential adverse effects. The ponds may be monitored at least weekly in April and May for egg masses or other signs of breeding. Once egg masses are found at each site (OU3, reference), the collection locations may be surveyed and up to 4 egg masses per species per area may be collected. The ponds may be revisited in late spring and early summer (May- early July) and up to 40 tadpoles per species per site in Gosner stages 21-25 and 37-40 may be collected and external development examined. Up to 20 metamorphosed juveniles may be collected per species per site later in July following full metamorphosis.

Final report must be submitted directly to Mike Hensler at the Libby Montana Fish, Wildlife & Parks (FWP) office at the same time the report is submitted to the Helena office.

The permit holder shall follow the department electrofishing guidelines attached to this permit.

When moving to or from all areas, collectors must take measures to disinfect and clean all gear

and equipment between waterbodies (see attached protocol). No live organisms can be transported away from the location of capture without FWP authorization.

REPORT: Upon expiration of the permit, please submit an electronic report containing the permit number, dates, location, and number and kind of specimens collected and released or killed. This form can be downloaded from the FWP website at <http://fwp.mt.gov/fishing> and emailed to beginnings@mt.gov.

By: 

Bruce Rich, Fisheries Bureau Chief
Montana Fish, Wildlife and Parks

Regional Fish Manager
c: Regional Fish Biologist

**ATTACHMENT D:
LIBBY OU3: PHASE V SAP, PART B, REV 0
AMPHIBIAN POND MONITORING DATA SHEET**

Date (mm/dd/yy): _____ Monitoring Personnel: _____

Field Logbook ID: _____ Field Logbook Page No: _____

Pond Identification:

Pond Name _____ Elevation _____

Pond GPS Coordinates: Latitude _____ Longitude _____

Weather Conditions (circle all that apply):

Precipitation: Rain Snow None

Atmospheric Conditions: Overcast Some Clouds Clear

Field Measurements:

Pond Water Temperature: _____

Pond Amphibian Presence and Identification:

Egg Mass Present (circle one): Yes No

Species	# Collected

ATTACHMENT D (cont)

Tadpoles Present (circle one): **Yes** **No**

Species	Gosner Stage	# Collected

Metamorphs Present (circle one): **Yes** **No**

Species	Gosner Stage	# Collected

Ok to collect with small tail bud

Abiotic Media Collections (for LA analysis):

Sediment Collection (circle one): **Yes** **No**

Surface Water Collection (circle one): **Yes** **No**

ATTACHMENT E
LIBBY OU3: PHASE V SAP, PART B, REV 0
FIELD SAMPLE DATA SHEET (FSDS)
SURFACE WATER AND SEDIMENT

[see Appendix C of the main document]

ATTACHMENT F
LIBBY OU3: PHASE V SAP, PART B, REV 0
POND SPECIMEN TALLY SHEET

Pond: _____

Species	Life Stage	Date	Sample Container #	Number Collected	Tally of # Collected
Tree Frog	Egg (n=4)				
	Premetamorphs (n=40)				
	Prometamorphs (n=40)				
	Metamorphs (n=20)				

Pond: _____

Species	Life Stage	Date	Sample Container #	Number Collected	Tally of # Collected
Spotted Frog	Egg (n=4)				
	Premetamorphs (n=40)				
	Prometamorphs (n=40)				
	Metamorphs (n=20)				

Pond: _____

Species	Life Stage	Date	Sample Container #	Number Collected	Tally of # Collected
Western Toad	Egg (n=4)				
	Premetamorphs (n=40)				
	Prometamorphs (n=40)				
	Metamorphs (n=20)				

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APPENDIX A.3
Protocol for the *In Situ* Fish Toxicity Studies
(Revision 1 – May 18, 2012)

APPENDIX A.3

Protocol for the *In Situ* Fish Toxicity Studies (Revision 1 – May 18, 2012)

Revision Log:

Revision No.	Revision Date	Revision Description
0	4/12/2012	---
1	5/18/2012	Change reference location from Bobtail Creek tributary [BTT] to Noisy Creek [NSY] (per Modification LFM-OU3-02).

1.0 Introduction

This protocol describes the standard operating procedures (SOPs) and sampling methods to be used when conducting *in situ* trout toxicity studies at Operable Unit 3 (OU3) and reference areas. The procedures established herein apply to both the eyed egg and juvenile trout *in situ* toxicity studies.

2.0 Health and Safety

All field personnel engaged in the conduct of these *in situ* trout toxicity studies must follow health and safety protocols described in their firm's health and safety plan (and that reflect the nature of the specific work being accomplished). Asbestos fibers are easily inhaled when disturbed and when embedded in the lung tissue can cause health problems. Significant exposure to asbestos increases the risk of lung cancer, mesothelioma, asbestosis (non-cancerous lung disease), and other respiratory diseases (ATSDR 2006). Accordingly, in addition to other health and safety procedures established in a firm's health and safety plan, all field personnel working on OU3 will wear protective garments and respiratory protection appropriate to HAZWOPER protection Level C. Respiratory protection will be via Positive Air Pressure Respirators (PAPRs).

3.0 Background

Historic mining and milling operations at OU3 have resulted in the release of Libby Amphibole (LA) to the environment, including surface water in lower Rainy Creek (LRC). Fish in LRC may be exposed to LA by direct contact with the contaminated surface water. As part of the Remedial Investigation at OU3, the United States Environmental Protection Agency (EPA) is pursuing multiple lines of evidence to evaluate if exposure of fish to LA in site surface water presents an unacceptable risk.

The EPA has previously investigated the question of toxicity to fish from LA in surface water through laboratory-based studies in which fish (rainbow trout fry) were exposed to LA in water derived from the site (Parametrix 2009). Although no adverse effects were observed, it was subsequently determined by EPA that LA in the water became clumped by organic material in the water, and that these clumps adhered to the walls of the exposure aquaria, reducing exposure concentrations to low levels. Subsequent attempts to solve this problem have not been successful. Consequently, this line of evidence did not provide meaningful results regarding the potential toxicity of LA on fish.

In the absence of the ability to reliably expose fish to LA under laboratory conditions, the OU3 Biological Technical Advisory Group (BTAG) determined that two (2) different *in situ* fish (eyed eggs and juvenile trout) exposure studies to natural waters in LRC containing LA represents would be conducted. These two studies represent appropriate alternative lines of evidence to help evaluate exposure and potential effects (if any) in fish given the observed limitations in conducting laboratory-based toxicity studies. The chief advantage of the *in situ* lines of evidence is that the exposure water will reflect actual site conditions. The chief disadvantage is that it is not possible to control LA concentration levels so it is unlikely that a dose-response relationship can be established for either study.

This protocol is focused on the specific methods and procedural details to conduct these *in situ* fish toxicity studies.

4.0 Definitions

Alevin: Term for a hatched fish

Eyed-egg: Early developmental stage of fish.

LA: Libby amphibole

NBF: Neutral buffered formalin

WVB: Whitlock-Vibert Box. Buried in a streambed or creek to hold fish eggs through hatch to swim-up.

Swoffer: Device that measures water velocity.

5.0 Responsibilities

This section presents a brief definition of field roles and the responsibilities generally associated with them. This list is not intended to be comprehensive and often additional personnel may be involved. Project team member information shall be included in project-specific plans (e.g., Sampling and Analysis Plan, etc.), and field personnel shall always consult the appropriate documents to determine project-specific roles and responsibilities. In addition, one person may serve in more than one role on any given project.

Project Manager: Selects site-specific sampling methods, sample locations, and constituents to be analyzed with input from other key project staff.

Quality Control Manager: Overall management and responsibility for quality assurance and quality control (QA/QC). Selects QA/QC procedures for the sampling and analytical methods, performs project audits and ensures that data quality objectives are fulfilled.

Field QA Officer: Performs audits of field activities being performed against procedures established in project SOPs for field work activities.

Field Team Leader (FTL): Implements the sampling program, supervises other sampling personnel. Also ensures compliance with SOPs and QA/QC requirements. Prepares daily logs of field activities.

Field Technicians (or other designated personnel): Assist the FTL in the implementation of field tasks. Perform the actual study activities, with the FTL, including study implementation, study set up, monitoring, sample collection, packaging, and documentation (e.g., sample label and log sheet, chain-of-custody record, etc.).

6.0 In Situ Toxicity Procedures

The remainder of this section contains a detailed summary of the equipment and procedures for conducting two (2) types of *in situ* toxicity studies using Westslope cutthroat trout (*Oncorhynchus clarki lewisii*): an eyed egg toxicity study (Section 6.1) and a caged juvenile trout toxicity study (Section 6.2).

6.1 In Situ Eyed Trout Egg Toxicity

The *in situ* trout egg toxicity test evaluates the toxicity of LA in OU3 creeks (with corresponding reference creeks) to eyed cutthroat trout eggs buried in creek redds during the rising hydrograph when LA fiber counts are expected to be maximized. The field portion of the study concludes a few days past the time when the alevins have all buttoned up (no visible yolk) and are in the initial days of swim up. The alevins

are then taken from the gravel to the lab where a swimming observation is completed. Alevins are then euthanized, preserved and examined externally (only) for any abnormalities, and then stored at the histologist's laboratory for any potential future use.

6.1.1 Equipment

The following is a list of equipment that will be needed to conduct the eyed trout egg *in situ* toxicity study.

- Global Position System (GPS) unit
- Flagging tape
- 35 Whitlock-Vibert egg boxes
- 18 stainless steel cages (14 x 7 inch) to house Whitlock-Vibert boxes
- Darice® size 7 plastic mesh (3 x 3 mm holes)
- 1000 Plastic zip ties
- Colored plastic tags
- Small (1-2" diameter) rigid PVC tubes, open on one end with a cap (perforated with small holes for water flow)
- Stainless steel bailers ($\leq 3/4$ inch)
- Small (thin) bottle brush (for water port cleaning)
- 80 surface water sample bottles (500-mL capacity, high-density polyethylene [HDPE] wide-mouth bottles to be provided by EMSL Analytical Inc. located in Libby)
- 1 yd³ 1.5 inch commercial, smooth gravel (for in the steel cages)
- 2 yd³ 5 - 8 inch commercial cobble / boulder
- Steel head rake
- Stainless steel shovel
- 400+ *Oncorhynchus clarki lewisi* eyed eggs obtained directly from Montana Fish Wildlife and Parks State fish hatchery
- 12 HOBO® TidbiT® submersible digital temperature loggers
- HOBO waterproof data shuttle
- Medium sized Tupperware® containers with lids
- Large sized Tupperware® containers with lids
- 4 large Rubbermaid® totes (with lid)
- Box of sandwich-sized zip-top baggies
- Box of large gallon size zip-top baggies
- Box of trash bags (Hefty® or equivalent)
- 2 small dipnets
- Package of 3 x 5 note cards
- Ruler with millimeter measure
- Handled scrub brushes (2 with sturdy plastic bristles)
- Water containers for creek water transport to offsite lab
- Small refrigerator (off-site lab)
- 2 ten (10) gallon glass aquaria with aerators and thermometers (off-site lab)
- Lab logbook (for off-site laboratory work)
- Field logbook (for field work)
- Digital camera
- Stop watch (or stopwatch function on mobile device)
- MS-222 (anesthetizing agent to euthanize fish)
- Disposable neoprene gloves
- Disposable scalpels
- Magnifying light
- Sharps container
- Top loading scale (100 g) with 0.01 g precision
- Top loading scale (400 g) with 0.1 g precision

- 2 gallons store-bought “spring” water
- Scale weights for daily calibration
- Detergent (soap)
- Plastic sheeting
- Paper towels
- Sharpie® brand markers (black)
- Ink pens
- Pre-filled with NBF plastic jars with lid
- 1 bottle of NBF to top off jars as needed
- Blank sample labels (adhesive) for NBF sample jars
- 160 Pre-printed sample labels for surface water samples (provided by EPA) (1 for sample bottle, 1 for FSDS)
- Chain of Custody Forms (carbon copy triplicate)
- 8 Manila file folders (for collecting forms and COCs)
- 144 Trout field observation data sheets (**Attachment A**)
- 450 Trout specimen processing forms (**Attachment B**)
- 1 or 2 Developmental Control Data Tracking Sheets (**Attachment C**)
- 12 Cage sample number tally sheets (**Attachment D**)
- 12 Swim observation data sheets (**Attachment E**)
- 80 Field Sample Data Sheets (FSDS) for surface water (**Attachment F**)
- Coolers
- Several rolls of clear packing tape (for affixing labels to surface water sample bottles)
- Strapping tape (coolers)
- Duct tape
- Custody seals

6.1.2 Whitlock-Vibert Box Preparation

Written permission will be obtained from Montana Fish Wildlife and Parks (MFWP) to obtain and use Whitlock-Vibert boxes (WVBs) in the OU3 and reference streams to be used in the *in situ* trout toxicity studies. The WVBs (35) will be obtained from the Federation of Fly Fishermen. This number allows over 100% above the actual needs of the study to support weekly cage cleaning activities and replacement in the event of breakage. All boxes (including extra's) will be modified to (1) add plastic mesh (Darice® size 7 plastic mesh, 3x3 mm grid size) to prevent alevin escapement; and (2) add a water sampling port.

Plastic mesh will be cut to fit within the WVBs (top, sides, bottom but not egg chamber bottom). Mesh will be cut to exact size of box interior and affixed with multiple plastic zip ties to ensure complete adherence and a flush mount (i.e., no buckling of mesh where alevins could escape or become trapped between mesh and cage). Colored plastic tags will be affixed to each box to provide a unique box identifier at each deployment location. This is necessary to support (1) WVB chamber water sampling box selection; (2) recording information on dead eggs/alevins that may be encountered in any given box during monitoring activities; and (3) for identifying the WVB in each location with an affixed temperature data logger and for WVB association of downloaded temperature data.

Rigid polyvinylchloride (PVC) tubes (small diameter), open on a single end (end exiting to creek; with a cap to fit snugly over the open end), and in a length sufficient to keep the open (but capped) end of the port well above the surface of the creek during periods of high water, will be installed in each WVB. The tube will be perforated (drilled) with small holes (i.e., to allow water flow into the tube from the surrounding chamber) over the length of the tube that is actually within the chamber inside the WVB. The tube is installed into the WVB by cutting a hole of sufficient diameter in the WVB lid and upper egg chamber floor to just accommodate the tube diameter (i.e., but not to allow alevin entrapment or escape). The overall length of the sampling port (tube) will be sufficient to sample water (without unburying the cages) from above the external cobble/boulder cover. This extended water sampling port will be fit with a snug cap to

keep surface water from entering the cage and will be removed only when WVB cage water is sampled (see Section 6.1.7).

One HOBO® TidbiT® submersible digital temperature logger will be affixed to the outside of one of the steel cages deployed in each stream region using secure fasteners (redundant to avoid loss of the logger). Temperature loggers will be affixed in a manner that facilitates easy downloading of data (once weekly during cage maintenance/cleaning).

6.1.3 Obtaining, Storing Eyed Eggs

Eyed Westslope cutthroat trout eggs (*Oncorhynchus clarki lewisii*) will be delivered to Golder in Libby, MT by a MFWP hatchery representative. Healthy eyed eggs are bright orange in color with a visible fish embryo within. Eggs will be maintained refrigerated in the off-site laboratory until ready for deployment in field WVBs (this is not expected to be more than a day or so).

Eggs will be used for filling WVBs and for establishing developmental (morphological) controls. Once eyed eggs are received, a Golder representative will count the eggs, remove any cloudy eggs, un-eyed or otherwise suspect eggs and discard. The eggs will be counted in batches of 30 into spring water (store bought) filled Tupperware containers to meet required deployment numbers (180 eggs total at 30 per WVB). All eggs are then stored in the refrigerator until actual deployment into the field. The total number of “good” eggs will be determined and written into the field log.

Any leftover eyed eggs (i.e., above and beyond the 180 needed to fill all the WVBs) will be divided into replicates of 30 each and kept in a spring water-filled Tupperware containers (no lid), marked with replicate numbers (Sharpie on side of container), in the refrigerator of the off-site laboratory. These eggs will represent “developmental” controls to assess overall egg quality and batch development. Developmental control eggs will be monitored twice weekly and the water changed (70% renewal) with fresh, store bought spring water (see Section 6.1.6). Temperature in the refrigerator will be monitored and adjusted weekly to reflect creek temperature measurements recorded from the warmest study creek (i.e., from data loggers). Developmental control egg observations (mortalities, etc), dissolved oxygen levels, refrigerator temperature change documentation, and other observations will be recorded on the Developmental Control Data Tracking Sheet prior to water change.

6.1.4 Creek Site Preparation for Whitlock-Vibert Installation

Creeks for deployment are as follows:

- OU3 Sites:
 - Lower Rainy Creek (LRC)
 - LRC-2 (this can be expanded as needed)
 - LRC-5 (this can be expanded as needed)
- Reference Sites:
 - Upper Rainy Creek (URC):
 - *Select only one (of the two) sample locations below for placement of URC WVBs:*
 - URC-1A (can be expanded as needed)
 - URC-2 (can be expanded as needed)
 - Noisy Creek (NSY)
 - NSY-R1 (can be expanded as needed)

The selected creek locations for WVB deployment (determined ahead of time from reconnaissance) should (ideally) be or approximate a natural redd (gravel/cobble area) that fish could use and be amenable to enhancement as needed using commercial gravel/cobble/boulder. Substrate at the candidate location is important; for example, the presence of smaller gravels along the edge of the thalweg in a given area suggests low disturbance during high flow events. Such a location would be ideal for WVB placement; tail out areas (end of riffle-run before a pool) may also be desirable areas for

deployment. The site is prepared by raking out a depression area along the sides of the selected deployment location, away from the deeper/faster thalweg, that will accommodate three (3) WVBs placed together and oriented perpendicular to creek flow. If necessary, structure (boulder/rock or log) can be added to create a breakwater area for placement that ensures flows are not excessive before WVBs are deployed. See also Section 6.1.5 for the WVB deployment strategy if three WVBs cannot be placed together anywhere within the desired creek reach segments (though this is the preferred strategy).

The bottom of the excavated deployment location for WVBs should contain a layer of natural (or commercial) spawning gravel on which the deployed cages will rest. The final depth of the deployment area, including the spawning gravel bottom, is such that the sides of the excavated area (which would be gravelly, cobble substrate) are approximately equal to the height of the closed steel cage containing the WVB (see 6.1.5). The excavated area is then allowed to sit undisturbed for 24 hours to flush away fine sediments prior to actual deployment of the WVBs containing the eyed eggs. All WVB deployment locations (i.e., in LRC; URC; NSY) are prepared in this manner.

It is recommended that all sites be excavated and prepared over a 2-3 day period (last week of April). Then over the course of 1 or 2 days, the WVBs with eggs can be deployed at each location.

6.1.5 Whitlock-Vibert Box Deployment

Modified WVBs are deployed into each creek site within 1-2 days following site preparation/excavation (Section 6.1.4). All WVBs should be in their respective creek gravel beds by no later than May 1- 2, 2012, to capture the rising hydrograph in the creeks (i.e., based upon historical data for LRC-6).

Three modified WVBs will be deployed at each of two locations in the vicinity of stations LRC-2 and LRC-5 (total of six (6) WVBs in LRC), and also at a single location in each of two (2) reference streams (URC, NSY). It is desirable to have the three WVBs buried in the same location in each creek location, if possible. If it is not possible to bury the three cages in a single location, then LRC will be divided into 3 segments (upper segment in vicinity of LRC-2, middle segment in between LRC-3 and LRC-4, and the third segment in the vicinity of LRC-5). Two WVBs will then be buried together in each of these three stream segments. Each location where WVBs are installed within LRC and reference streams (URC, NSY) will have GPS coordinates recorded. Flagging will be tied on a nearby tree (or stake) to denote deployment locations. The date and time of egg deployment at each location will be noted in the field logbook with deployment location (GPS) coordinates.

A total of 13 Tupperware containers, each with 30 eggs, are obtained from the offsite lab refrigerator and the containers are transported into the field in **complete and total darkness** inside a large lidded Rubbermaid Tote to avoid photo-sensitization and toxicity. Each of twelve (12) containers will be used to fill one of the 12 total WVBs to be deployed in OU3 and reference areas (eggs simply poured in). The remaining container (13th) will serve as a transport control (and will be marked as such on the container). In this manner, during WVB deployment a box of the eggs can simply be extracted and poured into the WVB without the need for counting.

To fill with eggs, place the open WVB into a Rubbermaid Tote filled with some of the creek water (to the level of the egg chamber). Pour in the eggs from one of the Tupperware boxes. The box is snapped securely closed and immediately placed into a 14 x 7-inch steel framed cage that contains a single layer of smooth, 1½-inch spawning gravel on the bottom (of the steel cage). The modified WVB is placed (centered) on top of this single layer of gravel and then more smooth spawning gravel is (quickly) filled in completely around the sides of the WVB (keep eggs out of direct light when filling boxes by shielding with your body). Spawning gravel is then placed inside the lid of the steel cage (largely filling it) and the cage lid is closed (water port extending through the steel bars) such that the WVB inside is now encased (top, sides, bottom completely full) with smooth spawning gravel (see Figure 1). Zip ties are used to secure the steel cage lid to the bottom of the steel cage on all sides (eggs are more protected from light in this environment). The filled steel cage can be set into the prepared area while the rest of the WVBs/cages are prepared for deployment.

Representative photos will be taken of WVB / egg deployment and a description (and camera frame number) of the photo(s) recorded in the field log book. The lid of the closed steel cage (water port extending through the cage bars above the surface of the creek water at high water periods) is secured to the steel cage bottom on all sides using plastic zip ties. Each WVB is prepared for deployment in this manner. Once closed, the steel cage can be placed in the creek water of the prepped site (awaiting burial) while other WVBs are being prepared for deployment.

Each steel cage and its WVB at a given deployment location will both have a unique color tag marked with the WVB identification number to uniquely identify the box and cage for purposes of data collection (i.e., water sampling, temperature reading, recording organism status, identifying appropriate redeployment location, etc). Numbers are assigned as discussed in Section 6.1.6.

Steel cages containing WVBs are placed in the prepared excavated area of each creek location on top of a layer of smooth spawning gravel and oriented perpendicularly to creek flow (again, outside of the thalweg) (see Figure 2). Gravel and cobble from the excavation area is then raked (using the steel rake) back to cover the sides of the steel cages (equal to the top of the steel cage lid) and then burial of the cages is completed by mounding cobble and boulder (5-8 inch size; a commercial source will be used to supplement) on top of the cages until no cage metal is visible (see Figure 3). Care must be taken not to damage the protruding, sealed PVC water ports of each cage when final burial occurs. Representative photos of the final burial locations at each deployment area will be taken at each creek and a description (and frame number) recorded in the field logbook. A sealed PVC water port, the same type as placed inside the WVB, is placed outside the box in the gravel on the upstream side of the boxes. This is to allow easy sampling of the pore water in the gravel (see Section 6.1.7, below).



Figure 1: Whitlock-Vibert placement within steel cages with smooth spawning gravel (photo courtesy of Nautilus Environmental)

Figure 2. Diagram of In-Situ Whitlock-Vibert Deployment

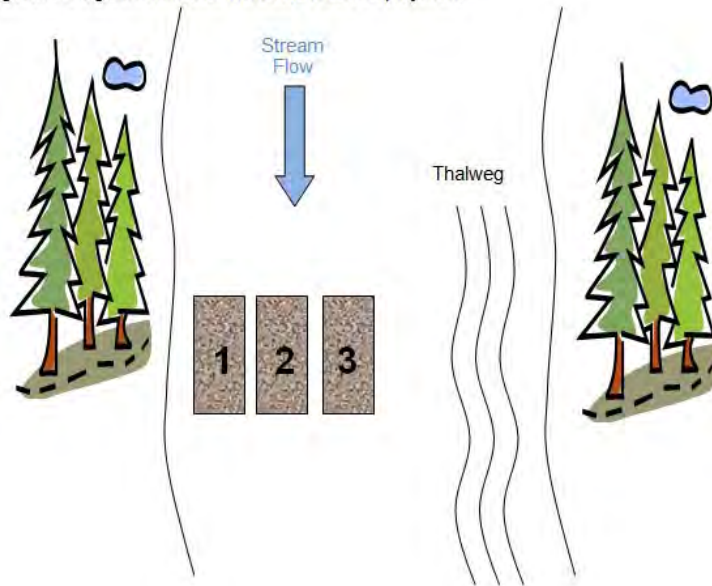


Figure 3: Whitlock-Vibert cages buried under cobble boulder
(Photo: Washington Department of Ecology)

6.1.6 Assigning Identification Numbers to WVBs

LRC. The regions in LRC where WVBs are deployed will be uniquely identified. If only two (2) regions are used for WVB deployment (see Section 6.1.5 for discussion of using 2 or 3 regions within LRC), the regions will be numbered as:

- LRC-2 (location in the general vicinity of LRC sample station 2), or
- LRC-5 (location in the vicinity of LRC sample station 5).

If three (3) sample stations need to be used in LRC to deploy all six (6) WVBs, then the regions will be numbered as follows:

- LRC-2 (in the general vicinity of LRC sample station 2)
- LRC-3 (in the general vicinity of LRC sample station 3)
- LRC-5 (in the general vicinity of LRC sample station 5)

URC, NSY. The locations in reference streams URC and NSY where WVBs are deployed will be uniquely identified. It is preferable that the three (3) WVBs be deployed at a single location in each reference stream, if possible, based on results of field reconnaissance (Note: this is to avoid having to put one (1) WVB in one region and two (2) in another; though if necessary this will be done as similarly discussed in Section 6.1.5 for LRC). Reference stream deployment locations are assigned as follows for URC and NSY:

- URC-1A: in the general vicinity of URC sampling station 1A
- URC-2: in the general vicinity of URC sampling station 2
- NSY-R1: in the general vicinity of NSY sampling station R1

Each box in a location will be marked on its colored tag with its WVB number to identify it uniquely from other WVBs in the same deployment location. In a given deployment region up to three colors will be used if three (3) boxes are deployed in that region (e.g., RD for red, GR for green, BL for blue, BLK for black, YL for yellow, OR for orange, etc). The same three colors can be used at all creeks since color alone does not identify a box. Box numbers must distinguish study type (WVB versus caged trout, CT) so that there is no confusion in distinguishing data for biological or water samples.

As an example, a WVB from URC-1A with a green tag and a WVB from LRC-2 with a red tag would have the following WVB identification numbers (respectively):

WVBURC1AGR and WVBLRC2RD

6.1.7 Whitlock-Vibert Cage Chamber Water Sampling

Water samples from inside the WVB chamber are collected to assess LA concentrations where the eggs are developing. This sampling occurs twice per week. Since one of these water collection days will coincide with a WVB cleaning activity (Section 6.1.8), it is important that the water sampling activity always occur BEFORE any cleaning activities are initiated to ensure the water sample is representative of the undisturbed condition within the chamber.

Two other types of water samples will also be collected at the time of routine WVB chamber water sampling on a limited frequency. These other water samples are as follows:

- Gravel pore water external to the WVB (just immediately upstream); and
- Overlying surface water in the WVB deployment location.

These two types of additional water samples are collected to evaluate the relationship in LA concentrations between the WVB chamber, the gravel pore water immediately external to the WVB and the overlying surface water (i.e., to support remedial decision-making).

Sample Bottles

Sample bottles (500-ml HDPE) will be picked up at EMSL in Libby and stored in the off-site laboratory for use. Prior to a water sampling day, field technicians should ensure all necessary sample bottles have pre-printed labels (provided by EPA) affixed with clear packing tape to each bottle so they are ready for use. Each bottle should also have a clear mark (Sharpie) denoting the approximate 400 ml level in the bottle; bottles should not be filled beyond this level. This is necessary to allow sufficient headspace in the bottles for sonication and UV/ozone disinfection activities that the analytical laboratory will conduct on each water sample.

Sampling Frequency:

LRC Locations:

Sampling of WVB chamber water will occur twice per week from a single box at each WVB location in LRC (e.g., LRC-2, LRC-5). If the study runs a total of 8 weeks in two LRC locations, this would translate to 32 water samples. If the boxes are distributed over 3 locations this would translate to 48 samples. Water sampling will occur on the same two days each week (e.g., Monday, Thursday). The WVB selected for water sampling during the first water sampling event will be randomly selected. Since all boxes in each LRC location (LRC-2, LRC-3, LRC-5) will be fitted with water sampling ports, water sampling events at each location should be regularly rotated among the boxes to ensure that all boxes are sampled as equally as possible over the course of the study.

In addition to the twice weekly WVB chamber water samples collected at each LRC location as noted above, two additional water samples will also be collected immediately upstream of the sampled WVB: an external gravel water port sample and an overlying surface water column sample. These two additional samples will be collected a total of twice from each LRC sample location (e.g., LRC-2, LRC-3, LRC-5): once per week for the first two weeks of the study (only). Thus, in week one of the WVB study, the selected WVB in each LRC location has its chamber water sampled plus an external gravel pore water and an overlying surface water sample. In the second week of the WVB study, these two additional samples will also be collected at each LRC location. They should be collected immediately upstream (matter of several inches or so) of the sampled WVB.

Assuming 3 sample locations in LRC, this translates to 6 additional water samples per week (3 external gravel pore water samples and 3 overlying surface water samples). Sampled over a two week period, this would equate to a total of 12 additional water samples (gravel pore water plus overlying surface water). After these additional samples have been collected in the first two weeks of the WVB study, the external gravel pore water sample ports can be removed from all LRC sample locations.

URC and NSY Locations:

Water sampling in URC and NSY will occur only once per week from a single box at both the URC and NSY WVB locations. Again assuming the study runs for 8 weeks in one location per reference stream, this would translate to 8 samples per creek or 16 total water samples over both reference creeks. The samples should be collected on the same day per week (e.g., Mondays). The box selected for water sampling during the first water sampling event will be randomly selected. Since all boxes will be fitted with water sampling ports, water sampling events at each location (URC, NSY) should be regularly rotated among the boxes to ensure that all boxes are sampled as equally as possible over the course of the study.

No external gravel pore water or overlying surface water samples are required in any of the reference creeks.

Assigning Sample Numbers to WVB Water Samples

All surface water samples (WVB chamber, external gravel pore water, overlying surface water) should be labeled using the pre-printed labels, with pre-assigned sample numbers (by EPA) that are specific to this study (see Section 8.5.1 of the main SAP/QAPP for details). For each sample collected a label is affixed to the actual sample bottle with clear packing tape and a second label is affixed to the FSDS.

Water Sampling Procedures

The 400 ml level should be pre-marked on all 500 mL HPDE water sample bottles and the bottles filled to this mark. Only a single WVB chamber is sampled at any given sampling location according to the frequency identified above. External gravel pore water and overlying surface water samples are also collected (in LRC only) at the frequency identified above. A stainless steel bailer is used to collect the samples. No more than 400 mL of water will be collected in the sample jar to ensure sufficient headspace is left in the bottle to accommodate ozonation / ultraviolet treatment performed by the analytical laboratory prior to analysis.

Sampling steps:

Note: Representative photos should be taken of water sampling with frame numbers and descriptions provided in the field logbook to illustrate this activity.

- Carefully remove the cap from the water port attached to the WVB;
- Insert stainless steel bailer slowly until it reaches the (closed) bottom end of the water port;
- Slowly raise the bailer (to minimize suction);
- Pour each bailer into the sample bottle until enough volume is collected (i.e., 400 mL);
- Place a pre-printed label on the outside of the bottle and cover with clear packaging tape;
- Put lid on sample bottle and close tightly;
- Record water sample number taken and date in the field logbook;
- Affix a corresponding sample number label on the surface water FSDS form (**Attachment F**);
- Rinse out bailer before next use;
- Place sealed, labeled sample bottle in cooler or transport box;
- For sampling LRC external gravel water ports, follow the same steps above (though at the lower specified sample collection frequency);
- For overlying surface water samples (LRC only for limited frequency specified above) fill bottle from just beneath surface of creek to the 400 mL mark on the bottle and put lid on bottle;
- Place bottles in cooler; and
- Take water samples (same day) to EMSL (Libby) for LA analysis.

6.1.8 Cleaning, Maintenance and Temperature Monitoring Activities

These activities involve both field and laboratory activities. Check that all necessary equipment for collection (dead eggs/alevins), downloading (temperature data), cleaning and maintenance activities is packed before leaving to conduct field activities.

Note: Representative photos of cleaning and maintenance activities should be taken and recorded in field logbook with frame number and description to illustrate activities.

Field Activities:

Once weekly (no more frequently to avoid stress to developing eggs) the WVBs at each location will be checked, observed and cleaned. Cleaning should never occur before WVB water sampling occurs, as discussed in Section 6.1.7. It is recommended that cleaning be done the same day each week (e.g., Mondays) so that exactly 7 days pass between cleanings (no more). Cleaning WVBs is done most efficiently by swapping out dirty WVBs with clean WVBs that are brought into the field so that field cleaning of cages is avoided and time that eggs are disturbed is minimized. WVBs deployed in a given creek should always be redeployed in the same creeks following cleaning to avoid exposing reference stream eggs/alevins to residual LA that may adhere to cleaned WVBs from LRC.

Eggs/alevins within the WVBs are transferred to the clean WVBs and data recorded weekly as follows:

- A Trout Field Observation Data Sheet will be completed for each WVB checked;
- All transference from one WVB to another is done within a large deep bin (e.g., Rubbermaid tote) that contains site water (avoids egg/alevin escapement to creeks);
- Begin by removing the overlying cobble/boulder from the burial location (care taken to not damage the extended water port);
- Remove the steel cage (care taken not to damage the extended water port), shielding cage from light with one's body as much as possible;
- Place steel cage in a water-filled bin and remove zip ties to open cage;
 - Remove the gravel into a medium Tupperware container for easy transference back into the cage;
- Remove the WVB, shielding from light with your body as much as possible, and place within the water within the bin;
 - If the steel cage has a temperature data logger another field technician should download the data using the HOBO data shuttle while the WVB is being processed
- Open the box and gently pour the eggs or alevins into a small, clear, creek water-filled Tupperware container (still working within the larger water-filled bin to avoid loss of eggs to the creek);
- Observe the physical condition (abnormalities, tumors, status of yolk, number of dead, etc) and movement (if alevins) of the organisms;
- Record condition of eggs/alevins and other observations for organisms on Trout Field Observation Data Sheet (**Attachment A**) (one data sheet per WVB checked)
 - Viewing the eggs/alevins from the bottom of the container can provide another view of yolk resorption; particularly as button up approaches.
- Remove any obviously dead eggs (white cloudy; healthy eggs are orange) or dead alevins (also white in color) and place (individually) in a zip-top bag labeled with date and WVB number in Sharpie (e.g., WVBLRC2BL; see Section 6.1.6 on WVB numbering convention) for later processing in the off-site laboratory;
- Record data on dead organisms on Trout Field Observation Data Sheet;
- Open up a clean WVB (same color tag as steel cage) and submerge the WVB into the creek water within the Rubbermaid bin and pour the eggs/alevins gently from the Tupperware container into the WVB. Shield from light at all times;
- Snap close lid on WVB;
- Replace the WVB into the steel cage on top of a thin layer of the gravel;
- Repack the rest of the steel cage (and lid) with the same spawning gravel. Be careful to not damage the extended water port during re-packing and closure of the cage (**WVB should not be visible through the spawning gravel in the steel cage**);
- Use the plastic zip ties to re-close the fully packed (with spawning gravel) steel cage;
- Replace the steel cage in the burial area and once all WVBs have been replaced and repacked, rebury all with the smooth cobble / boulder;
- Place "dirty" WVBs into a large zip-top bag labeled with stream segment location (LRC-2, URC-1 for later cleaning and redeployment at same location);

- Replace any broken or damaged WVBs / steel cages; and
- Move on to the next location for maintenance, following the same procedures.

As embryos button up, yolk status should be carefully checked in each WVB during the weekly check. When 50% or more of the alevins appear to have buttoned up (no longer any visible yolk) in a location, study termination (removal of WVB from gravel for behavioral testing) is likely to be a matter of days away. Cages will need another check and once all alevins are 2-3 days past full yolk resorption study termination planning should begin in earnest.

Lab Activities

There are three off-site laboratory activities to be completed following WVB field maintenance:

- Processing of dead eggs / alevins, recording information, and assigning sample numbers using Trout Specimen Processing Forms (**Attachment B**);
- Cleaning of dirty WVBs collected from field; and
- Checking/recording data on developmental controls (this to be done twice weekly) using the Developmental Control Data Tracking Sheet (**Attachment C**; kept in folder next to refrigerator in off-site laboratory)

Processing Dead Field Organisms:

Any dead eggs/alevins collected from the WVBs should be processed and information recorded on Trout Specimen Processing Form (**Attachment B**) as follows:

- One Trout Specimen Processing Form per organism
- Remove dead organism from labeled zip-top bag; Blot moisture off on a folded paper towel;
- Weigh organism to nearest 0.01 g on calibrated scale
- If alevin, measure snout to tail length (millimeters) using a ruler
- Fill in specimen weight, length and other required information on Trout Specimen Processing Form, including WVB Sample Number assigned (see below for sample number assignment procedures)
- Drop egg or alevin (whole) into a small, labeled (with WVB sample number and process date) NBF-filled jar
- Screw lid down tightly and store NBF-filled container in a cooler in laboratory for later shipment to Northwest ZooPath for examination (and storage)
- Discard bag(s) in trash

Assigning Sample Numbers to Eggs and Alevins

Sample numbers assigned to all eggs/alevins will include:

- WVB identification number
- Developmental stage
 - EG = egg
 - AL = alevin
- Date (mm/dd/yy)
- Number of organisms

For example, 2 dead eggs from a red WVB at LRC-5 collected on May 11, 2012 would have the following sample numbers:

WVB-LRC5-RD-EG-051112-1
WVB-LRC5-RD-EG-051112-2

One dead alevin from a green WVB in URC-2 collected on June 1, 2012 would have the following sample number:

WVB-URC2-GR-AL-060112-1

Since there are a total of 30 specimens in each WVB it may be useful to maintain a cage sample number tally sheet (**Attachment D**) to keep track of sample numbers assigned over the course of the study for each cage. This form can ensure that sequential sample numbers for a WVB (or caged trout, CT) are accurately assigned; including a description of whether the organism was euthanized or found field dead. **Attachment D** contains a cage sample number tally sheet that can be copied and used for each of the 24 cages (12 WVB, 12 caged trout) that will be tracked with specimen sample number assignments during the course of the *in situ* trout studies.

Cleaning WVBs

Dirty WVBs are also cleaned at the off-site laboratory using a stiff bristle brush and clean water only (no soap) to remove algae and other fouling materials in the cage (external and internal mesh). Using the thin bottle brush, the water sampling port should also be gently cleaned and rinsed within each WVB to remove any fouling material. Water used to clean and rinse cages will be disposed of in designated waste water containers located near the off-site lab. Clean WVBs are lightly dried (paper towels) and placed back into the labeled (i.e., same creek where it was removed and will be redeployed) large zip-top bag.

Maintenance and Processing of Developmental (Refrigerator) Controls

These controls are submitted to the pathology laboratory for archival (only) with other study specimens since their purpose is just for assessing egg quality during the course of the field study.

Developmental controls in the off-site laboratory refrigerator must be checked and maintained twice weekly. Completed activities for developmental controls are as follows:

- Record control maintenance activities on the Developmental Control Data Tracking Sheet (**Attachment C**) (kept in folder next to refrigerator)
- Check downloaded temperature data logger information for all creeks to identify the warmest creek (must be identified)
 - Once identified, when 2 weeks of downloaded temperature data is available for “warmest creek”, review and determine how much warming is occurring. Use this information to determine a **twice weekly adjustment to refrigerator temperature** to keep developmental controls roughly on par with development of eggs in the gravel at the warmest creek.
 - New temperature logger data collected weekly on the shuttle for warmest creek will be used to update and refine the control refrigerator temperature adjustments
 - Download HOBO temperature data on shuttle to a laptop regularly to back up the data;
- Change water in each developmental egg control replicate (70% renewal only rather than 100% renewal) using store-bought spring water.
- Lightly feed alevins (if lacking yolk; use food obtained from MFWP)
- Discard used water in waste containers outside of the laboratory
- Remove any dead organisms from each of the replicate developmental control boxes
- Observe developmental controls remaining in each replicate and record brief observations on Developmental Control Data Tracking Sheet

- For any dead developmental control organism:
 - Measure fresh weight (to 0.01 g) and record in laboratory notebook (*no need for use of Trout Specimen Processing Form for these organisms since these samples are strictly for field use in gauging egg development success in the field*)
 - If alevin, measure length (in millimeters) from snout to tail tip and record in laboratory notebook with an assigned number (Developmental Control, Rep 1, #1; Developmental Control, Rep 2, #1, etc)
 - Place each individual egg/alevin directly (no incisions) in a small, labeled (as in bullet above) NBF-filled container (no sample number assigned)
 - Screw cap down tightly
 - Place labeled bottles in a cooler for storage at the lab for later submission and storage (only) at Northwest ZooPath (developmental controls are archived with other study samples but are not examined).
- Repeat refrigerator temperature adjustments, and observations of developmental controls, every four days (twice weekly).

6.1.9 Study Termination and Fish Swimming Observations¹

Morphological stage of the developmental controls will provide an idea when study termination is approaching (but not exactly). This will vary based upon creek temperature (for example, URC is colder and expected to lag behind eggs in LRC and NSY in terms of morphological development). As this time approaches, the off-site laboratory will be made ready to start the swimming observation tests.

Swimming observation tests are conducted on alevins a few days after swimup (i.e., a few days after all alevins have lost all visible (external and internal) yolk). At this point, the WVBs must be pulled out of the gravel for swimming observations. Pulling WVBs for swim observations is predicated on the developmental stage of the alevins (a few days past all yolk resorption). Therefore, though ideally the first WVBs to have their swimming observed would be from a reference site (e.g., NSY), this may or may not be possible if there are delays in development across the creeks. All boxes will be checked twice a week (or more frequently if needed) in the last week of the study to confirm the developmental stage of alevins in each creek and gauge when boxes will be pulled for testing. If possible, it is desirable to have the first swimming observations be from a reference creek (e.g., NSY) so that “normal” swimming is identified first.

All swimming observations should be conducted as quickly as possible once WVBs are pulled from the gravel.

[Note: it is possible that the timing of alevin swim observations at WVB study termination may overlap with termination of caged juvenile trout; if this poses logistical challenges the caged trout can wait an extra day or two for termination since these fish can be regularly fed. In this way, all testing proceeds smoothly and efficiently and minimizes chances for error or unnecessary loss of test organisms].

Pulling WVBs from Gravel:

No more than 3 WVBs at a time should be pulled and taken to the offsite lab for swim testing to ensure alevins do not stay out of gravels for more than a few hours before behavioral testing and euthanization.

¹ These observations do not truly represent a behavioral test but an observation of an activity---swimming. A true behavioral “challenge” cannot be offered to these field organisms given study time constraints and considering field laboratory setup and equipment constraints; both of which are critical to establishing baseline behavior and to identify subsequent deviations from said baseline based on the “challenge” of interest.

Steel cages removed from each region to be tested at off-site laboratory are placed in a large Rubbermaid tote/bin containing fresh creek water. Cover the tote with lid for transport to off-site laboratory. Keep cages covered in creek water in the bin, in darkness, while awaiting testing.

Sufficient creek water will be collected at this time from the creek and also transported to the off-site laboratory, with the WVBs, to support filling 10-gallon test aquaria in the off-site laboratory and to refresh water of awaiting alevins in remaining steel cages/WVBs. Up to 30 or 35 gallons of creek water is recommended.

NOTE: Aquaria used for testing OU3 fish should be marked as OU3 (Sharpie) and dedicated to only testing OU3 fish; and similarly for the aquaria used for reference creek fish.

All aquaria are emptied, cleaned and well rinsed with clean water following each WVB test.

Alevin Acclimation and Swim Test Setup

In the off-site laboratory, two (2) Golder scientists will be present at all times to prepare and conduct all testing. A third person may be needed as a “runner” to gather other WVBs and associated creek water. Acclimation and testing is conducted as follows:

- Bring tote containing creek water and alevins in steel cages/WVB into the lab.
- Fill a 10-gallon aquarium with additional creek water of the creek being tested (one 10 gallon aquarium is dedicated as an OU3 creek aquarium and the second 10-gallon aquarium is dedicated as a reference creek aquarium);
- Turn on aerator to a gentle aeration rate;
- Write (largely and legibly) the WVB identification number (e.g., WVBNSY1GR) with a Sharpie marker on a 3 x 5 note card;
- Place note card on table in front of the filled aquarium to identify alevins to be observed (and visible to camera for photos);
- Hold WVB to be tested inside of the aquaria and open the box to release the alevins;
- Start a 15 minute timer (acclimation period before swimming observations begin);
- On another table covered with plastic sheeting, set up a large, labeled Tupperware container with creek water and add appropriate concentration of MS-222 (fish anesthetizing agent that humanely euthanizes the fish);
 - This may be done right before fish come out of aquaria for euthanizing;
 - Cover this Tupperware container with lid when not being used (it can be reused until no longer effective in euthanizing);
- Note WVB identification number to be tested on Trout Swimming Observation Data Sheet (**Attachment E**) ;
- Calibrate weighing scale (and make record in a laboratory notebook); and
- When the 15-minute acclimation period has past, the swimming observations will begin.

Swimming Observations:

Swimming observations are not associated with any specific “challenge”. Fish are simply observed in the tank and “normal and abnormal” swimming activity is noted over discreet time intervals (2, 10, 20 and 30 minutes) within the 30 minute observation period. Observations are recorded on the Trout Swimming Observation Data Sheet (**Attachment E**).

Note: Fish are very likely to be stressed given the change from dark field conditions to testing conditions, which involve much more light and the presence of people in the testing area (lab). Stressed fish may swim very fast (race around) within the aquaria and may breathe at accelerated rates.

Representative photos of laboratory and test set-up, showing WVB number, should be taken and descriptions recorded in the laboratory notebook with camera frame number and a description to illustrate activities.

- When the 15-minute acclimation period has past, set the timer for 30 minutes and start the timer;
- Take representative photos of the test in progress (showing note card with WVB number showing in frame)
- Note camera frame numbers in laboratory notebook;
- Record swimming observations on Swim Observation Data Sheet (**Attachment E**) at the 2, 10, 20 and 30 minute time intervals noted.
- At conclusion of 30 minutes finish filling in observation form and begin processing of alevins;
- Second technician to assist in processing and data recording to ensure all alevins are swiftly processed and aquaria can be cleaned and set up for the next WVB test.

Post Swim Test Processing of Alevins

Note: some representative photos of post test processing activities should be taken and camera frame number, description placed in laboratory notebook.

- Clean neoprene gloves
- Trout specimen processing forms (one per specimen; 30 total per WVB)
- Remove a small group of alevins from aquarium using dip net for processing (leaving others in the aquaria just tested);
- Place this group in Tupperware container with water and MS-222 to be euthanized;
- When alevins are dead, remove individually with forceps and place on folded paper towel to blot off excess moisture;
- Assign a WVB sample number individually to each alevin (see Section 6.1.8 above on how to assign sample numbers; there will be organism numbers ranging up to 30 given the number of alevins in a single WVB and the Cage Tally Sheet can be used to assist in accurately assigning sample numbers and to ensure all organisms in a cage get sample numbers assigned);
- Weigh each individual alevin to nearest 0.01 g;
- Using a ruler taped to table (with millimeter measure) measure alevin length (in mm) from snout to tail
- Record alevin weight and length on Trout Specimen Processing Form (**Attachment B**);
- Place each alevin whole in a small, labeled (with WBV sample number and process date) NBF-filled container and close lid tightly;
- Once every fish in a subgroup is euthanized, weighed and measured, repeat processing steps with all remaining alevins in test tank;
- Remove aerator and empty 10 gallon aquaria into waste water tank located outside of lab;
- Rinse aquaria with clean water several times and empty rinse water into waste water tank;
- Dry aquaria with paper towel to ready for use again (reattach aerator);
- Place labeled NBF-filled containers in a cooler (since preserved no ice needed).
- Start process using all the steps above for the remaining steel cages/WVBs awaiting testing and processing (beginning with a 15-minute acclimation period);
- When all WVB cages have been processed, including URC (which is likely to lag developmentally), coolers will be inventoried and COC forms filled in and double checked;
- Coolers with NBF jars are packed (including packing material to keep jars from moving around inside cooler) and then shipped to NorthWest ZooPath via UPS;
- All fish will be retained until otherwise directed by study Principal Investigator, Sue Robinson, Golder Associates (i.e., for possible future use).

6.1.9 Biweekly Data Submissions to EPA

Digital photos taken should be downloaded to a laptop as a secondary backup from the camera. Photos should be uploaded every two weeks to the OU3 eRoom.

If possible, bi-weekly data uploads will occur to the OU3 eRoom by scanning and uploading pages from the field logbook, fish forms (Attachments A through E), water FSDS forms (Attachment F), lab notebook pages, HOBO temperature data and digital photos. This is accomplished by going to the WR Grace office in Libby with a laptop, scanning the required pages and forms using the office copy/scanner and then uploading the data to the following web address: <https://team.cdm.com/eRoom/mt/LibbyOU3>. Individual eRoom access codes are assigned to Golder staff working on the project and these access codes will be used by individuals uploading data.

Questions or problems with data uploading to the OU3 eRoom should be directed to Lynn Woodbury: woodburyl@cdmsmith.com (303) 383-2382.

6.2 *In Situ* Caged Juvenile Trout Toxicity

The *in situ* juvenile cutthroat trout toxicity test evaluates the toxicity of LA in OU3 creeks (with corresponding reference creeks) during the falling hydrograph when creek velocities from snow melt are expected to be declining, but concentrations still at a higher level than during typical low water periods. The study includes a swimming behavior observation at study conclusion. Euthanized, preserved juvenile trout are examined externally (only) for any abnormalities and then stored at the histologist's laboratory for potential future use (if any).

6.2.1 Equipment

The following is a list of equipment that will be needed to conduct the caged juvenile *in situ* toxicity study.

- Global Position System (GPS) unit
- Flagging tape
- 13 floating fish boxes (15" x 15" x 13") (0.042 m³)
- 1/4 inch metal mesh
- Wood for construction of frame and lid
- Pipe insulation for padding bottom of cages
- Hardware (stainless hinges, latches, screws, etc)
- 48 Buoys (4 per cage)
- Cable for attaching buoys
- Colored plastic tags
- Swoffer meter
- T-bars for anchoring cages to creek bed
- Cables to tether cages to land structure (to avoid loss of cages)
- 200+ *Oncorhynchus clarki lewisi* juvenile fish (3-5 inches) obtained directly from Montana Fish Wildlife and Parks State fish hatchery
- 36 500-mL capacity, high-density polyethylene [HDPE] wide-mouth bottles to be provided by EMSL Analytical Inc. located in Libby) (Surface water sample bottles)
- 72 Pre-printed sample labels for surface water samples (provided by EPA) (1 for bottle; 1 for FSDS)
- 500 Trout field observation data sheets (**Attachment A**)
- 360 Trout specimen processing forms (**Attachment B**)
- 12 Cage sample number tally sheets (**Attachment D**)
- 12 Swim observation data sheets (**Attachment E**)
- 36 Field Sample Data Sheets (FSDS) for surface water (**Attachment F**)
- 8 Manilla file folders (to hold cage fish forms and COCs)
- Fish food (assume provided by hatchery)

- Large sized Tupperware containers with lids
- Sandwich sized ziplock baggies
- 2 dipnets
- 3 x 5 note cards
- Handled scrub brush with soft bristles to gently scrub netting
- Water containers (carboy) for creek water (swim testing)
- 2 20-gallon glass aquaria with aerators and thermometers (off-site lab)
- Lab notebook (same as for WVB)
- Ruler (with millimeter measure)
- Field logbook (same as for WVB)
- Digital camera
- Stop watch (or stopwatch function on mobile device)
- MS-222 (anesthetizing agent to euthanize fish)
- Disposable neoprene gloves
- Disposable scalpels
- Magnifying light
- Sharps container
- Top loading scale (100 g) with 0.01 g precision
- Top loading scale (400 g) with 0.1 g precision
- Scale weights for daily calibration
- Detergent (soap)
- Plastic sheeting
- Paper towels
- Sharpie brand markers (black)
- Ink pens
- Small, medium sized NBF-filled containers
- Blank sample labels (adhesive) for NBF sample jars
- Several rolls clear packing tape for placing pre-printed sample labels on sample bottles
- Chain of Custody Forms (carbon triplicate)
- Coolers
- Custody Seals
- Strapping tape

6.2.2 Floating Box Preparation

Box dimensions are 15 inches length x 13 inches width x 13 inches height. The interior volume equals approximately 0.042 cubic meters (2535 cubic inches, 1.467 cubic feet).

Boxes will be constructed as shown in Figure 4 using wood, ¼ inch metal or plastic meshing, floats and hardware (hinges, latches, cable for buoy attachment, etc). The bottom of the cage will have a layer of foam pipe insulation added to the frame to protect the bottom of the cage frame. The goal is to deploy 3 cages at a single location in each of two reference creeks and 3 cages in each of two locations in LRC. This translates to a total of 12 cages for deployment in the study. Each box in each deployment region will have a uniquely colored tag with cage identification number written (in black Sharpie) that is clearly visible (see Section 6.2.7 on assigning cage identification numbers).

Boxes will have a tag identifying local contact information and scientific collection permit number.

Representative photos will be taken of constructed cages prior to deployment and camera frame numbers / descriptions provided in the field logbook.



Figure 4 Floating juvenile trout cage (Photo courtesy of Montana Fish Wildlife and Parks).

6.2.3 Obtaining, Storing Juvenile Fish

Juvenile Westslope cutthroat trout (*Oncorhynchus clarki lewisi*), approximately 3-5 inches in length, will be delivered to Golder in Libby, MT by a MFWP hatchery representative. Approximately 200 fish will be provided (enough to install 15 fish per cage over a total of 12 cages) with a few extra to cover any incidental mortality that could occur during transport to Libby.

A sampling of 15 of the fish in this batch will be weighted, measured and data recorded in the laboratory notebook. This information provides a relative measure of the “starting” weight and length of the batch of fish provided by the hatchery (note: individual measurements will not be taken of each fish at study start as this is not practical and will stress the fish too much). If there are enough fish to deploy 15 in each of the 12 cages, then these measured fish can then be euthanized and discarded as they represent starting weights and measures only. If there are not sufficient fish numbers, then these weighed and measured fish will be separated (water-filled Tupperware container) and then randomly deployed (equally) among both OU3 and reference cages (i.e., in the event these fish introduce a treatment bias from handling).

Representative photos of delivered fish will be taken and camera frame number and description provided in the field logbook.

6.2.4 Site Preparation for Floating Box Installation

Creeks for deployment of floating cages are as follows:

- OU3 Sites/regions:
 - Lower Rainy Creek (LRC)
 - LRC-2 (this can be expanded as needed)
 - LRC-5 (this can be expanded as needed)
- Reference Sites/regions:
 - Upper Rainy Creek (URC):
 - *Select only one (of the two) sample regions below for placement of URC floating cages:*
 - URC-1A (can be expanded as needed)
 - URC-2 (can be expanded as needed)
 - Noisy Creek (NSY)
 - NSY-R1 (can be expanded as needed)

The selected creek locations for caged trout (CT) deployment (determined ahead of time from reconnaissance) should be in a pool, as close as is reasonably possible, downstream from the WVB deployment locations. In this manner, temperatures monitored at WVBs can be used as reasonable surrogates for the temperature in the creek pools where floating cages will be deployed, and to keep exposure conditions as spatially similar as is possible. The selected pool location is likely to require modification to construct an artificial breakwater behind which cages can be safely floated (but that does not cut off water flow) without risk of excessive stream velocity. A Swoffer velocity meter will be used to regularly check that flow velocities above where cages are deployed is maintained at or below <0.75 ft/s, the critical velocity limit. This avoids creating excessive swimming stress on the juvenile fish that could result in mortality. If/when velocities approach the critical velocity, additional structure will be added to reduce water velocity below the critical velocity.

It is recommended that selected pool locations, identified from reconnaissance, be modified with breakwaters (structure of logs/boulders) over a 2-3 day period just before mid-May (i.e., just before the hydrograph begins to drop around May 15th based on historical LRC-6 data) and when boxes must be installed. Velocities will be measured daily from this point on (in and outside the cages) through the end of the 30-day study to ensure cages are always maintained in an environment where creek flow velocities during high water are below the critical level. T-bars can be installed in a row upstream of all deployed cage locations to serve as debris catchers (e.g., branches, long sticks) to safe guard cages.

Representative photos of each deployment site, including breakwater features (if any) will be taken and camera frame number and descriptions put in the field logbook.

6.2.5 Floating Cage Deployment

For planning purposes, all cages will be deployed in all the creeks by May 14-15, 2012, (assuming fish are received within this time period from MFWP) to catch the estimated lowering hydrograph. Swoffer measures upstream of any constructed breakwater will give an indication of when creek flows are dissipating (and if breakwater modifications may be needed to ensure flow velocities are not too low).

As noted in Section 6.2.4 above, three (3) floating cages will be deployed in the nearest downstream pool to the deployed WVBs in the reach region as is possible. If, as noted in Section 6.1.5 above relating to deployment of WVBs, three (3) locations (instead of two) in LRC are necessary, then the same deployment strategy will be followed for deploying the floating cages (i.e., 2 floating cages in an upper, middle and lower region of LRC). Each location where floating cages are installed within LRC and reference streams (URC, NSY) will have GPS coordinates recorded in the field logbook. Flagging will also be used to denote deployment locations.

Juvenile fish, brought to Libby by the Montana Fish Wildlife and Parks State hatchery, will be immediately deployed. Those fish that cannot be immediately deployed will be transported to offsite lab and maintained in 20 gallon aquaria, with aerators, until ready to be deployed into the cages at LRC, URC and NSY. Temperature in the holding aquaria will be maintained at 52 degrees Fahrenheit (based on MFWP recommendation) while fish are maintained in the aquaria (if it is necessary to hold the organisms prior to deployment). This time should be as short as possible to avoid stress to the fish (the goal is to immediately take the fish to cages once delivered and deploy).

To deploy fish in the field cages, a dip net will be used. Fish that are maintained in the off-site aquarium (if any) will be placed in a large, lidded, Rubbermaid tote filled with water for transport to deployment sites (i.e., unless the fish can be immediately taken to deployment locations in the containers provided by Montana FWP State Hatchery for cage placement). Fish will be transported to each deployment location and placed into the cages by counting out a total of 15 fish into a lidded (to ensure no escapement to creek if dropped), water-filled Tupperware container. The Tupperware container is walked carefully (to avoid slipping or tripping) over to the floating cage, the wooden lid lifted and the fish gently poured into the cage. The wooden lid is then lowered and secured. This is repeated until each cage at each deployment location contains 15 fish. Fish deployment date and time should be noted in the field logbook at each location. Remaining fish will be deployed at all locations (OU3, reference) in this manner.

Representative photos of fish deployment should be taken and camera frame number and description provided in the field logbook.

6.2.6 Cleaning, Maintenance and Monitoring Activities

These activities involve both field and laboratory components. Check that all necessary equipment for data collection (e.g., fish condition, collection of dead fish), cage cleaning, feeding is packed before leaving to conduct field activities.

Field Activities

Daily throughout the 30-day study duration, each cage will be checked and data recorded on a Trout Field Observation Data Sheet (**Attachment A**). These data include: swoffer (velocity) readings inside and outside each cage; dissolved oxygen and temperature. At this time fish will also be fed and data recorded on fish condition (including mortalities) on a Trout Field Observation Data Sheet (**Attachment A**). Any dead fish will be removed, placed in a baggie and labeled with cage identification number and date (in Sharpie), for processing back at the off-site laboratory. Adjustments to breakwater structures (if any) may be necessary to keep velocity below the critical velocity of <0.75 ft/s. Other cage or deployment site condition issues (e.g., apparent tampering, etc) will also be recorded daily in the field logbook by cage identification number.

Fish will be fed daily by scattering food within the cage. The food source and amount will be as recommended by the Montana Fish Wildlife and Parks State hatchery. It is anticipated that this will be fish pellets.

The outside of the cages will be cleaned daily if needed, by gently removing anything trapped against the outside netting (sticks, etc) and brushing the mesh sides if needed with a bristle brush. Given size of the mesh there should be no need to remove fish waste from cages (which will drop through).

Laboratory Activities

The only laboratory activity associated with cleaning maintenance and monitoring activities for the caged juvenile trout study is the processing of dead juvenile trout (if any) that may be collected during daily cage checks, as follows:

- Don clean neoprene gloves;
- Remove dead fish from labeled zip-top bag;
- Blot moisture off on a folded paper towel;
- Assign sample number following convention below;
- Measure length of fish (in millimeters) using a ruler;
- Weigh organism to nearest 0.01 g on calibrated scale;
- Record data on Trout Specimen Processing Form (**Attachment B**)
- Using a pair of forceps, a disposable scalpel and a magnifying light make a ventral incision from mandible to cloacae for proper preservation
- Place "opened" fish in a labeled (with sample number and process date) NBF-filled jar; use a Cage Sample Number Tally Sheet (**Attachment D**) to ensure all sample numbers from a cage are properly assigned (1 through 30)
- Screw lid down tightly and store labeled NBF-filled container in a cooler in the lab (no ice needed as organisms are preserved) for later shipment to Northwest ZooPath;
- Place used, disposable scalpel in sharps container;
- Discard bag in trash; and
- Repeat for each dead fish collected.

The NBF-filled containers will later be shipped to Northwest ZooPath for examination (and storage).

6.2.7 Floating Cage Water Sampling

Sample Bottles

Sample bottles (500-ml HDPE) will be picked up at EMSL in Libby and stored in the off-site laboratory for use. Labels with pre-printed sample identification information will be affixed to each sample bottle (labels provided by EPA). Another label is affixed to the FSDS. Water sampling will always occur before feeding or cage cleaning activities occur to minimize debris in the samples.

Sampling Frequency:

LRC Locations:

Sampling of caged fish pen water for total LA will occur twice per week from a single cage at each location in LRC (e.g., LRC-2, LRC-3, LRC-5). The box selected for water sampling during the first water sampling event will be randomly selected. Thereafter, water sampling events at each creek region should be regularly rotated among the cages to ensure that all boxes are sampled as equally as possible over the course of the study. Sampling should occur on the same days each week (e.g., Monday, Thursday). Note: Sampling should always occur before cage cleaning is undertaken to ensure a representative water sample, free of added debris, is collected. Assuming an exposure duration of about 4 weeks for the caged fish study, this will generate 8 water samples per location in LRC. If the LRC cages are located in 2 locations, this will result in 16 total water samples. If the LRC boxes are distributed across 3 locations, this will result in a total of 24 water samples.

URC and NSY Locations:

Water sampling in URC and NSY will occur only once per week from a single box at both the URC and NSY cage locations. The box selected for water sampling during the first water sampling event will be randomly selected. Thereafter, water sampling events at each creek region should be regularly rotated among the cages to ensure that all boxes are sampled as equally as possible over the course of the study. One water sample will be collected from a single cage from each reference area (NSY, URC) weekly. Assuming an exposure duration of about 4 weeks, this will represent a total of 4 water samples per reference creek. With two reference creeks, this represents a total of 8 water samples. Water samples should be collected the same day each week (e.g., Mondays).

Assigning Cage Identification Numbers for Caged Trout (CT) Study

LRC

The locations in LRC where cages are deployed will be uniquely identified. If only two (2) locations are used for cage deployment (see Section 6.2.5 for discussion of using 2 or 3 regions within LRC), the locations will be numbered as:

- LRC-2 (location in the general vicinity of LRC sample station 2), or
- LRC-5 (location in the vicinity of LRC sample station 5).

If three (3) sample locations need to be used in LRC to deploy all six (6) cages, then the locations will be numbered as follows:

- LRC-2 (in the general vicinity of LRC sample station 2)
- LRC-3 (in the general vicinity of LRC sample station 3)

- LRC-5 (in the general vicinity of LRC sample station 5)

URC, NSY

The regions in reference streams URC and NSY where cages are deployed will be uniquely identified. It is preferable that the three (3) cages be deployed at a single location in each reference stream, if possible, based on results of field reconnaissance (Note: this is to avoid having to put one (1) cage in one region and two (2) cages in another; though if necessary this will be done as similarly discussed in Section 6.2.5 for LRC). Reference stream deployment locations are assigned as follows for URC and NSY:

- URC-1A: in the general vicinity of URC sampling station 1A
- URC-2: in the general vicinity of URC sampling station 2
- NSY-R1: in the general vicinity of NSY sampling station R1

Each cage in a region will have its cage identification number marked and clearly visible on a colored tag that uniquely identifies it from other cages in the same deployment location. In a given deployment region/location up to three colors will be used if three (3) boxes are deployed in that region (e.g., RD for red, GR for green, BL for blue, BLK for black, YL for yellow, OR for orange, etc). The same three colors can be used at all creeks since color alone does not identify a cage. Cage numbers must distinguish study type (WVB versus caged trout, CT) so that there is no confusion in distinguishing data for biological or water samples.

As an example, a cage from the caged trout (CT) study from URC-1A with a green tag and a CT cage from LRC-2 with a red tag would have the following cage identification numbers (respectively):

CTURC1AGR and CTLRC2RD

Assigning Sample Numbers to Caged Trout Water Samples

All surface water samples will be labeled using the pre-printed labels with pre-assigned sample numbers (by EPA) specific to this study. Water sample labels will be provided by EPA (see Section 8.5.1 of the main SAP/QAPP for details).

Surface Water Sampling From Cages

Only a single cage is sampled at any given sampling location according to the frequency identified above. Sampling will occur by taking a grab sample using a 500-ml HDPE bottle with the 400 mL line pre marked on each bottle (Sharpie). Water sampling will always occur before any cage cleaning or feeding activities to ensure the sample is free from debris.

To sample:

- Remove the lid from the sample jar;
- Downstream and outside of the cage, rinse the sample jar several times with creek water and discard;
- Raise lid on selected cage and gently immerse the sample bottle to collect the sample, being careful to not disturb fish or trap fish in a sample bottle;
- Fill the bottle to the pre-marked 400 mL line on the bottle (this leaves headspace for lab processing activities);
- Screw on lid tightly to seal.
- Affix a pre-printed sample number label to the outside of the bottle and cover with clear packaging tape;
- Affix a corresponding label on the FSDS
- Make note in the Field Logbook of the sample number and date;
- Place sample bottle in cooler or transport box; and

- Transport water samples (same day) to EMSL in Libby for LA analysis.

Assigning Sample Numbers to Juvenile Trout Specimens

Sample numbers reflect much information and therefore, dashes are used in between information fields to ensure clarity.

Sample numbers assigned to all juvenile trout will include the following information:

- Cage identification number
- Specimen identifier (JT for juvenile trout)
- Date (mm/dd/yy)
- Organism number (ranges from 1 – 15)

For example, 2 dead juvenile trout from a red floating cage (CT) at LRC-5 collected on June 11, 2012 would have the following unique assigned sample numbers for specimen processing:

CT-LRC5-RD-JT-061112-1 CT-LRC5-RD-JT-061112-2

Sequential trout processed after this date (either because dead in the field or at study termination) will be similarly and sequentially numbered such that the final trout processed in a cage group will have an organism number of 15.

It is recommended that a cage tally sheet be filled in for each cage to keep track of sample numbers over the course of the study (and minimize sample assignment errors). The cage tally sheet (**Attachment C**) for each cage should be kept in the off-site laboratory and updated daily with the number of dead fish (if any) and so that sequential organism sample numbers can be assigned throughout and at the conclusion of the study.

6.2.8 Study Termination and Fish Swimming Observations

As the end of the 30 day study duration approaches, the laboratory will be set up and made ready to start the juvenile trout swimming observation tests.

[Note: timing of juvenile trout test termination and need for swimming observations may overlap with termination of WVB study termination. If this poses a conflict, termination of (swim tests) with the caged trout will be delayed an extra day or two since these fish can be fed (i.e., priority is given to WVB termination testing first). In this way, all aspects of study termination and final testing proceed smoothly and efficiently for both types of studies, minimizing chances for error.]

Collecting Juvenile Trout from Floating Cages:

If possible, the first cages pulled should be from a reference creek so that juvenile fish swimming behavior from uncontaminated creek water can be observed first. After the first reference creek swim testing is completed then the second reference creek cages will be pulled and tested. Cages at LRC will be the last to be pulled and fish taken to the off-site laboratory for swim observations.

To transport fish to the off-site laboratory for testing, one person holds a large water-filled Tupperware container, while a second person uses a dip net to capture fish from the floating cage and place them in the Tupperware container (Note: the Tupperware container with fish from the cage is always held over the opening of the cage in case it is inadvertently dropped, to prevent juvenile fish from escaping into the creek and being lost). Once all fish have been removed from the cage, the lid is placed firmly on the Tupperware container and it is immediately transported to the off-site laboratory (along with at least 20 gallons of creek water for swim testing). Each cage is harvested and transported to the off-site laboratory for testing in this manner. When all three

cages from a location have had their fish harvested, tested and euthanized, juvenile trout from the next location will be collected and brought to the off-site laboratory for testing, proceeding with all reference creeks first.

Juvenile Fish Acclimation and Swim Test Setup

In the off-site laboratory, 2 Golder scientists will be present at all times to conduct the preparation and testing. Other staff may be needed as “runners” to gather other cages/juveniles and collect creek water.

Acclimation and swim observation test setup will be conducted as follows:

- Bring Tupperware container with collected juvenile trout into the laboratory;
- Don clean neoprene gloves;
- Fill a 20-gallon aquarium with additional creek water of the creek being tested;
- Turn on aerator to a gentle aeration rate;
- Write (largely and legibly) the cage identification number (e.g., CTNSYR1GR) with a Sharpie marker on a 3 x 5 note card
- Place note card on table in front of the filled aquarium to identify juvenile trout to be observed;
- Gently, pour the juvenile trout slowly and gently into the aquarium
- Start a 15 minute timer (acclimation period before swimming behavior observations begin); Obtain a Swim Observation Data Sheet (**Attachment E**);
- On another table covered with plastic sheeting, set up a large Tupperware container with creek water, and add appropriate concentration of MS-222 (fish anesthetizing agent that humanely euthanizes the fish);
 - This may be done right before fish come out of aquaria for euthanizing;
 - Cover this Tupperware container with lid until ready for use;
- Calibrate weighing scale (and make record in a laboratory notebook); and
- When the 15-minute acclimation period has past, the swim observations will begin.

Swimming Observations

These observations are not associated with any specific “challenge”. Fish are simply observed in the tank and swimming observations are noted over a 30-minute period.

Two (2) Golder scientists will be in the off-site laboratory at all times when testing is being conducted to support conduct of swim behavior observations and post-processing.

- When 15-minute acclimation period has past, set the timer for 30 minutes and start the timer;
- Take representative photos of the test in progress (showing note card with CT number showing in frame);
- Note camera frame numbers in laboratory notebook;
- Though observations are continuous during the 30 minute test, observations are noted on the Swim Observation Data Sheet at the four (4) specified time intervals (2, 10, 20 and 30 minutes);
- At conclusion of 30 minutes, begin post-test processing of fish; and
- Repeat the process above for the next cage.

Post Swim Test Processing of Juvenile Trout

- Trout Specimen Processing Forms (one per specimen)
- Don clean neoprene gloves;
- Remove fish using dip net in small groups for processing (leaving others in the aquaria just tested);
- Place this group in Tupperware container water and MS-222;

- When fish are dead, remove individually with forceps and place on folded paper towel to blot off excess moisture;
- Assign a CT sample number to each fish (see further above; there will be organism numbers ranging up to 15 given the number of fish in a cage); (use Cage Sample Number Tally Sheet, Attachment D)
- Weigh each individual fish to nearest 0.01 g
- Using a ruler taped to table (with millimeter measure) measure fish length (in mm) from snout to tail;
- Record fish weight and length on Trout Specimen Processing Form (Attachment B);
- Once a fish is weighed and measured, using a disposable scalpel, and working under magnifying light, make a ventral incision from mandible to cloacae to open up the fish for internal preservation;
- Place each fish in a labeled (with CT sample number and processing date) NBF-filled jar;
 - Repeat steps for the remainder of the fish in the small euthanized group
- Repeat all steps above until all fish remaining in the aquaria have been euthanized and individually processed;
- Place used scalpel in sharps container;
- Place labeled NBF-filled jars in a cooler for later shipment to pathology laboratory (since preserved no ice needed);
- Remove aerator and empty 20-gallon aquaria into waste water tank located outside of laboratory;
- Rinse aquaria with clean water several times and empty rinse water into waste water tank;
- Dry aquaria with paper towel so ready for use again (reattach aerator).
- Either go to the field and collect another cage for testing (preferred), or if a field runner has already done this, begin the juvenile trout acclimation and swim test setup steps identified above using the second clean, 20-gallon aquarium (OU3 fish only in the dedicated OU3 aquaria; reference fish only in the dedicated reference aquaria).
- All caged juvenile trout are tested and processed in this manner.
- When all cages have been processed, coolers with NBF-filled containers will be inventoried and COC forms filled in and double checked (they are now ready to be shipped);
- Coolers with NBF jars are packed (including packing material to keep jars from moving around inside cooler) and then shipped to NorthWest ZooPath via UPS;
- Pathology laboratory will examine preserved fish externally (only) for any signs of abnormality.
 - Unusual lesions (if any) will be noted.
 - No histology will be conducted as part of this study.
- Results will be summarized in a brief report provided to Principal Investigator (Sue Robinson)
- All study methods and findings (including report from histology lab) will be summarized in a data report provided to EPA
- All fish will be retained by Northwest ZooPath until otherwise directed by study Principal Investigator (i.e., for possible future use).

6.2.9 Data Backup and Biweekly Data Submissions to EPA

Digital photos taken should be downloaded to a laptop as a secondary backup from the camera. Photos should be uploaded every two weeks to the OU3 eRoom.

Bi-weekly data uploads to the OU3 eRoom will be completed by scanning and uploading pages from the field logbook, fish forms (Attachments A through E), water FSDS forms (Attachment F), laboratory notebook pages and digital photos. This is accomplished by going to the WR Grace office in Libby with a laptop, scanning the required pages and forms using the office copy/scanner and then uploading the data to the following web address: <https://team.cdm.com/eRoom/mt/LibbyOU3>. Individual eRoom access codes are assigned to Golder staff working on the project and these access codes will be used by individuals uploading data.

Questions or problems with data uploading to the OU3 eRoom should be directed to Lynn Woodbury: woodburyl@cdmsmith.com (303) 383-2382.

7.0 References Cited

Agency for Toxic Substances and Disease Registry (ATSDR). 2006. Asbestos Exposure and Your Health.

ATTACHMENT A

**LIBBY OU3: PHASE V SAP, PART B, REV 0
TROUT STUDY FIELD OBSERVATION DATA SHEET**

WVB or Cage Number: _____ Date: _____ Sampler: _____

Cage Site GPS Coordinates: Latitude _____ Longitude _____

Field Logbook ID: _____ Field Logbook Page No: _____

Field Measurements:

Caged Trout:

Swoffer (Velocity) Readings: Upstream _____ Inside cage: _____

Dissolved Oxygen: Outside cage _____ Inside Cage _____

Water Temperature: _____

Whitlock Vibert Boxes:

Ambient Water Temperature (°C): _____

Dissolved Oxygen: _____

Condition of Eggs / Alevins / Trout:

Dominant Developmental Stage (circle): Eyed Eggs Alevins Juvenile Trout

Condition (circle one): Normal Abnormal (Description): _____

#Dead Organisms: _____

General Fish Behavior (circle one):

Unremarkable Remarkable

If Remarkable, brief comments: _____

Alevins w/ Complete Yolk Re-sorption (circle one): None Few Half Most All

If "half" or "most" circled, study end may be near for this cage and more frequent cage checks needed)

ATTACHMENT B

**LIBBY OU3: PHASE V SAP, PART B, REV 0
TROUT SPECIMEN PROCESSING FORM**

Specimen Identification:

Cage Type (circle one): WVB CT

Specimen Type (circle one): Egg Alevin Juvenile Trout

Cage Number: _____

Assigned Specimen Sample Number¹: _____ Process Date: _____

Egg / Alevin / Trout Specimen Description (circle one):

Dead in Field Euthanized (@ Study Termination)

If Found Dead in Field, Describe Specimen Condition (circle one):

Unremarkable

White / Cloudy

Malformation / Abnormality (brief description): _____

Specimen Physical Data:

Weight (to nearest 0.01 g): _____

Length (millimeters): _____

WVB = Whitlock-Vibert Box CT = Caged Trout

¹ Follow WVB or CT study procedures for assigning sample numbers as discussed in *In Situ* Trout protocol.

ATTACHMENT C

LIBBY OU3: PHASE V SAP, PART B, REV 0 EYED EGG DEVELOPMENTAL CONTROL DATA TRACKING SHEET **Checks Done At Least Twice Per Week**

Name of Creek for Developmental Control Temperature Sync: _____

Date	Water DO (mg/L)	Refrig. Temp (° F)	Sync Creek Average Weekly Temp (° F) ²	Revised Refrig. Temp (° F) ³	70% H ₂ O Change? (Y / N)	Feed Alevins? (Y / N)	# Dead per Replicate	Observations

² From once weekly creek temperature probe data download. Refrigerator temperature adjustment done only once or twice weekly (twice if more than a couple of degree difference is needed to avoid shocking eggs)

³ Refrigerator temperature setting should not be changed more than a few degrees at a time.

ATTACHMENT D

LIBBY OU3: PHASE V SAP, PART B, REV 0 CAGE SPECIMEN SAMPLE NUMBER TALLY SHEET

Cage Type: WVB CT (circle one)

Cage Number: _____

Organism No ⁴	Assigned Sample Number	Field Dead or Euthanized Organism (FD or E)?
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		

⁴ No more than 30 organisms with sample numbers per Whitlock-Vibert Box. No more than 15 organisms with sample numbers per (floating) trout cage.

17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		

ATTACHMENT E

LIBBY OU3: PHASE V SAP, PART B, REV 0 TROUT SWIMMING OBSERVATION DATA SHEET

WVB or Cage Number: _____

Test Date: _____

Observer Name: _____

Fish Observations

(Note the Approximate Number of Fish Engaging in Behavior During Each Time Interval)

Behavior	2 mins.	10 mins.	20 mins.	30 mins.
Fish swimming very fast around tank ¹				
Fish on bottom of tank (vertical orientation with snout up or down) ²				
Fish static in water column, not sinking ²				
Fish moving slowly in water column ²				
Fish "lying" on cage bottom				
Fish floating on side (no movement)				
Fish having difficulty maintaining vertical/horizontal orientation				
Fish swimming in circles				
Other "unusual" behavior (describe)				

Notes:

1 – Could be temporary stress indicator

2 – Normal behaviors

ATTACHMENT F

LIBBY OU3: PHASE V SAP, PART B, REV 0 FIELD SAMPLE DATA SHEET (FSDS) SURFACE WATER AND SEDIMENT

[see Appendix C of the main document]

APPENDIX A.4
Protocol for the Resident Trout Field Collection Study
(Revision 0 – April 12, 2012)

APPENDIX A.4

Protocol for the Resident Trout Field Collection Study

(Revision 0 – April 12, 2012)

1.0 Introduction

This protocol describes the standard operating procedures (SOPs) and sampling methods to be used when collecting and processing resident trout at Operable Unit 3 (OU3) and reference area collection sites.

2.0 Health and Safety

All field personnel engaged in the conduct of these trout studies must follow health and safety protocols described in their firm's health and safety plan (and that reflect the nature of the specific work being accomplished). Asbestos fibers are easily inhaled when disturbed and when embedded in the lung tissue can cause health problems. Significant exposure to asbestos increases the risk of lung cancer, mesothelioma, asbestosis (non-cancerous lung disease), and other respiratory diseases (ATSDR 2006). Accordingly, in addition to other health and safety procedures established in a firm's health and safety plan, all field personnel working on OU3 will wear protective garments and respiratory protection appropriate to HAZWOPER protection Level C. Respiratory protection will be via Positive Air Pressure Respirators (PAPRs).

3.0 Background

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including surface water and sediment in lower Rainy Creek (LRC). Fish in LRC may be exposed to LA by direct contact with the contaminated surface water or sediment. As part of the Remedial Investigation at OU3, EPA is pursuing multiple lines of evidence to help evaluate if exposure of fish in LRC to LA in presents an unacceptable risk.

The first line of evidence that EPA has investigated consisted of demographic studies of fish density in LRC compared to that in appropriate reference streams. This line of evidence suggests that the density of trout in LRC is lower than expected by comparison to upper Rainy Creek (URC) and other nearby reference streams (Noisy Creek, Bobtail Creek tributary), and that this difference is statistically significant (Parametrix 2009a, 2010). In particular, the density of small fish (< 65 mm), which are usually considered to be young of the year, appears to be much lower in LRC than in reference streams. Efforts to determine whether these differences are attributable to habitat differences or to an adverse effect of LA have not been definitive (Parametrix 2009a). Hence, additional information is needed to determine if the apparent reduction in fish population in LRC may be related to LA or whether the reduction is best understood in terms of habitat.

A second line of evidence that EPA investigated with the goal of resolving this question consisted of laboratory-based studies in which fish (rainbow trout fry) were exposed to LA in water derived from the site (Parametrix 2009b). Although no adverse effects were observed, it was subsequently determined that LA in the water became clumped by organic material in the water, and these clumps bound to the walls of the exposure aquaria, reducing exposure concentrations to low levels. Subsequent attempts to solve this problem have not been successful. Consequently, this line of evidence does not provide meaningful results regarding the potential toxicity of LA on fish. As an alternative to laboratory exposures, *in situ* exposures of caged fish will be implemented in 2012.

In the absence of the ability to reliably expose fish to LA under laboratory conditions and because of the inherent limitations of *in situ* exposures, the OU3 BTAG determined that examination of resident fish

captured from LRC for the occurrence of external lesions is an appropriate and potentially useful additional line of evidence to help with the assessment of potential risk to fish.

This protocol is focused on the specific methods and procedural details regarding the field collection of resident trout from OU3 and reference streams and examination for possible lesions. The chief advantage of this line of evidence is that the exposure media (surface water and sediment in OU3 creeks) will reflect actual site exposure conditions. A disadvantage is that it is not possible to control LA concentration levels so it is unlikely that a dose-response relationship can be established.

4.0 Definitions

LA: Libby amphibole

GPS: Global Positioning System

NBF: Neutral buffered formalin

YOY: Young of the year

5.0 Responsibilities

This section presents a brief definition of field roles and the responsibilities generally associated with them. This list is not intended to be comprehensive and often additional personnel may be involved. Project team member information shall be included in project-specific plans (e.g., Sampling and Analysis Plan, etc.), and field personnel shall always consult the appropriate documents to determine project-specific roles and responsibilities. In addition, one person may serve in more than one role on any given project.

Project Manager: Selects site-specific sampling methods, sample locations, and constituents to be analyzed with input from other key project staff.

Quality Control Manager: Overall management and responsibility for quality assurance and quality control (QA/QC). Selects QA/QC procedures for the sampling and analytical methods, performs project audits and ensures that data quality objectives are fulfilled.

Field QA Officer: Performs audits of field activities being performed against procedures established in project SOPs for field work activities.

Field Team Leader (FTL): Implements the sampling program, supervises other sampling personnel. Also ensures compliance with SOPs and QA/QC requirements. Prepares daily logs of field activities.

Field Technicians (or other designated personnel): Assist the FTL in the implementation of field tasks. Perform the actual study activities, with the FTL, including study implementation, study set up, monitoring, sample collection, packaging, and documentation (e.g., sample label and log sheet, chain-of-custody record, etc.).

6.0 Resident Trout Field Collection Study Procedures

The remainder of this section contains a detailed summary of the equipment and procedures for conducting the field collection of resident trout.

6.1 Equipment

The following is a list of equipment that will be needed to conduct the resident trout field collection study.

GENERAL

- Global Position System (GPS) unit
- Clipboard
- Field logbook
- Three-ring binder to store necessary forms used to record and track samples collected at the site. Binders will contain the Fish Information Forms.
- Rite In The Rain® paper
- Permanent marking pen – used to record information in field notebooks and data sheets.
- Digital camera
- Lab notebook (processing)

ELECTROFISHING

- Backpack electroshocker unit
- Spare Battery
- Waders
- Buckets – for holding fish
- Wader belts
- Fiberglass handled dip nets
- Block nets (if needed)
- Trash Bag - used to dispose of gloves and any other non-hazardous waste generated during sampling.
- Plastic (e.g., Gladware®) containers

FISH TRAPPING

- Modified minnow traps (see Figure 1)
- Salmon egg bait
- Whirlpaks (perforated)
- 1:100 betadyne to water solution

FISH PROCESSING

- Ruler with millimeters
- Digital camera
- MS-222 (anesthetizing agent to euthanize fish)
- Disposable neoprene gloves
- Roll of wax paper
- Top loading scale (0.01 g precision)
- Scale weights for daily calibration
- Plastic sheeting
- Paper towels
- Plastic (e.g., Gladware®) containers
- Sharpie brand markers (black)
- Ink pens
- NBF pre-filled plastic jars with lids
- Extra NBF
- Disposable scalpels
- Sample labels (adhesive)
- OU3 Chain of Custody (COC) Forms (carbon copy triplicate)
- Scientific collection permit (Attachment A)
- Fish Information Form (Attachment B)
- Coolers
- Strapping tape
- Custody seals

6.2 Study Design

The data needed for this line of evidence includes measurement of the nature and frequency of external lesions (if any) in resident trout collected from on-site (OU3) area creeks compared with that for resident trout of the same species collected from one or more reference creeks.

Three species will be targeted for capture in the reference and OU3 streams, to the extent available in the field:

- Cutthroat trout
- Rainbow trout
- Trout hybrids (cutbow)

Two size classes of resident trout are the focus of the study. At present, there are no data to indicate whether there is any important age-dependence in the potential effects of LA on fish. However, given that the density of small fish (< 65 mm size class), which are usually considered to be young of the year (YOY), appears to be much lower in LRC than in reference streams, this study will collect fish in the < 65 mm age class. The study will also target collection of a second size class of fish > 65 mm - < 100 mm because some YOY may grow larger than the <65 mm earlier in the season depending on water temperature and other habitat factors; which could explain their original absence from the 2008 / 2009 fish density studies. Based on typical growth rates, it is expected that cutthroat trout could reach this size range by about mid-August, and rainbow trout could reach this size somewhat earlier. Based on this, the optimum time for fish collection is early to mid-August. The study will likely take 7 – 10 days to complete.

Collected fish will be sent to a histology lab for external examination. Tumors or abnormalities will be excised, embedded, stained and examined histologically. All preserved fish collected as part of this study will be maintained by the histology laboratory in the event that more detailed histological preparation and microscopic examination may be needed in the future. For example, if other studies at the site suggest that there are effects on the growth or survival of fish in LRC, then a more detailed analysis for external or internal lesions that might explain these effects may be appropriate.

6.3 Collection Locations

On-Site Sampling Locations

Three streams exist in OU3 that may be impacted by LA: Carney Creek, Fleetwood Creek, and LRC. Of these, the best and most extensive fish habitat is present in LRC, and fish population data have been collected for this reach (Parametrix 2009a, 2010). In addition, concentrations of LA in LRC tend to be the same or higher than in Carney Creek or Fleetwood Creek. Consequently, LRC is the optimum location for collection of resident fish to determine potential external lesions which may be associated with LA exposure.

Within LRC, capture should occur at multiple stations, including the locations where previous population density studies have been performed (TP-TOE2, LRC-1, LRC-2, LRC-3, and LRC-5). To the extent feasible, approximately equal numbers of fish should be collected at each station to help ensure the data set is spatially representative of LRC and is not unduly influenced by any individual station. To this end, the length of each sampling reach may be increased as needed to increase the likelihood of capture at a given station.

A creek reconnaissance in LRC will be conducted immediately ahead of the study (early to mid-August) to identify expanded creek station reaches and support logistics and deployment activities.

Reference Sampling Locations

Three streams are potentially useful as reference locations: Upper Rainy Creek (URC), Noisy Creek, (NSY-R1), and Bobtail Creek tributary (BTT-R1). In selecting which of these is/are most appropriate for use in this study, matching on species of fish present is thought to be most important. Fish density studies that have been performed to date indicate that fish in LRC consist of rainbow, cutthroat and cutbow (hybrid) trout. URC contains mainly cutthroat trout, NSY-R1 contains mainly cutthroat and cutbow trout, and BTT-R1 contains rainbow and brook trout. On this basis, BTT-R1 is not considered optimal for use as reference because of potential species differences, but both URC and NSY-R1 are potentially valuable and both of these two reaches will be used for collection of reference resident trout. Station reaches in URC and NSY-R1 can be increased in length as needed to increase the likelihood of capture.

A creek reconnaissance will be conducted in URC and NSY-R1 immediately ahead of the study (early to mid-August) to identify expanded creek station reaches and support logistics and deployment activities

6.4 Sample Collection Goals

Any fish collected will be under the auspices of a scientific collection permit, obtained from Montana Fish Wildlife and Parks (MFWP) (Attachment A)). The goal of the collection program is to collect fish in two size classes with the following total numbers identified as collection goals:

1. < 65 mm : 10 fish per creek, approximately equal numbers per station if possible
2. > 65 mm - < 100 mm: 10 fish per creek, approximately equal numbers per station if possible

This will equate to a total of 20 fish per creek, or a total of 60 fish over all three creeks.

6.5 Field Sampling Methods

Fish will be collected both by electro-shocking, block nets (as needed) and modified minnow traps. Modifications to minnow traps will include reducing the size of the opening so only small sized fish (<65 mm, >65 mm - < 100mm) can enter, and adding a smaller mesh over the trap to ensure the targeted fish cannot escape through the sides. The electro-shocking unit will be a backpack type with positive current. The electro-shocking operator is trained for this activity in the State of Montana.

Ten traps will be set in each stream reach. Traps will be baited with salmon eggs (previously disinfected for 10 minutes with 1:100 betadyne to water solution) held in perforated "whirlpaks" (Bryant 2000). Traps will be placed for ½ to full day (daylight hours only) at each sample station reach within each creek (these locations can be expanded as needed to increase capture potential). Traps will be retrieved in the order that they were set. If sample numbers are not met in the initial round of trapping, trapping will be repeated for a second day as needed at each location. Therefore each sample station reach will be trapped over a total of no less than two (2) trap days.

Fish collected by each method will be recorded on the Fish Information Form to species (if known) and length recorded using a fish measuring board (goal is to get 10 fish in each creek in each of the two size classes <65 mm and > 65 mm - < 100 mm). Information on external parasites or other external gross abnormalities will be recorded. Measurements and observations will be recorded on the Fish Information Form (Attachment B). Fish lengths will be verified during processing at the offsite lab. Any fish caught that are not within the required size needs will be released downstream of the reach (below block net if needed).

Collected fish will be kept in cold site creek water (i.e., small Gladware container labeled by creek reach) until transported to the off-site laboratory for processing and preservation.

6.6 Processing Field-Collected Trout in the Off-site Laboratory

All fish will be processed prior to shipment to the histology laboratory, where they will be examined (externally) in detail. Processing in the off-site laboratory includes recording fresh weights of each fish and preserving the organism for examination by the histopathologist.

Fish collected in the creeks (LRC, URC, NSY-R1) are processed in the off-site laboratory. It will be most efficient (and reduce errors) by having one laboratory technician assigning sample numbers and recording data (lab notebook, Fish Information Form) while the other technician conducts the measurements and euthanization.

Processing fish is generally as follows:

Fish Euthanization

- In a large-size Gladware container, place one liter of spring water (grocery store bought; NOT distilled water)
- Weigh out 250 mg of MS-222 on a piece of wax paper
- Pour MS-222 into water and cover with lid
- Gently stir and/or shake from side to side until fully dissolved
- Remove lid
 - Note: keep MS-222 mixture covered with lid when not in use
 - A fresh batch of MS-222 is mixed when a batch is no longer effective in euthanizing
- Place weighed organism in MS-222 solution until no longer moving
- Remove fish (gloved hands) and dab dry with paper towel to remove excess solution

Obtain Fish Weight

- Clean neoprene gloves
 - Gloves may not be interchanged between processing reference site (URC, NSY-R1) and treatment (LRC) fish to minimize cross contamination (always use clean gloves)
- See Section 6.7 on how to assign sample numbers
- Gently dab fish on clean paper towel to remove excess moisture
- Record sample number of specimen to be weighed on the wax paper square
- Place wax paper square on calibrated scale and tare to zero
- Place fish on the scale and record weight (to nearest 0.01 gram)
- Record weight in lab notebook (by sample number)
- Record sample number and weight on the Fish Information Form

Zero out scale before next weighing and use fresh wax paper for each specimen weighed

Fish Length Measurement (verification of field-collected measurements)

- Tape ruler firmly to table top
- Measure fish length from snout to end of tail (in millimeters, mm)
- Record length (in mm) and compare to length on the Fish Information Form. Correct (line out and write in correct value) as needed.

Fish Preservation

- Record fish sample number on the label of an NBF-filled container (with lid)
- Using lighted magnifying glass, make an incision from the mandible to anus (ventral side)
- Place opened euthanized fish into the NBF-filled container and tightly close the lid.
- Place sealed, labeled NBF-filled container in a cooler (to go to Northwest ZooPath)
 - Because fish are preserved there is no need for ice in the cooler
- Repeat euthanizing, weighing, measuring, incising, preserving and sample number recording procedures for all fish
- Place used scalpel in sharps container.

Quality Control

When all fish in a batch have been processed, lab technicians will cross check the number of NBF-filled specimen containers in the cooler against (i) the number of fish sample numbers recorded in the lab notebook (with weight and length measures), and (ii) against the length and weight measures recorded on each Fish Information Form. Recorded information in lab notebook and Fish Information Forms should match exactly. The number of entries in the lab notebook, the number of entries in the Fish Information Forms and the number of NBF-filled bottles in the cooler should match exactly. In this manner any missing or inaccurate measurements or data recorded in lab notebook or Fish Information Form can be immediately rectified.

6.7 Assigning Fish Sample Numbers

This will be done during processing at the off-site laboratory. Sample numbers are recorded on sample jars containing NBF, in the lab notebook and on any Fish Information Form. Sampling numbers will be individually assigned to each collected fish considering the following information:

Creek Name/Reach:

LRC (Lower Rainy Creek):

- TP-TOE2 (considered LRC)
- LRC-1
- LRC-2
- LRC-3
- LRC-5

URC (Upper Rainy Creek):

- URC-1A
- URC-2

NSY (Noisy Creek):

- NSY-R1

Species: CT (cutthroat); RB (rainbow); CB (cutbow); UK (unknown¹)

Age Class: A = < 65 mm
B = > 65 mm - < 100 mm

Date: Collection date (mm/dd/yy)

Examples of Assigning Sample Numbers:

Sample numbers include creek and reach; species, size class, date and the number of the fish that fit this description (in the event multiple fish of a species and size category are caught in a particular creek reach).

Thus, two (2) < 65 mm cutbow trout collected on August 18th from Lower Rainy Creek in the vicinity of LRC-5 would have the following sample numbers assigned:

LRC5-CB-A-081812-1
LRC5-CB-A-081812-2

¹ Fish < 65 mm are difficult to identify to species and may only show par markings. If a fish species is not known, it will be identified as UK (unknown).

Note the number -1 at the end of the sample number designates the number of organisms caught (first is “1”, second is “2”, etc).

A single cutthroat trout in the >65 mm - <100 mm size class collected from Upper Rainy Creek on August 16th near URC-1A would have the following sample number assigned:

URC1A-CT-B-081612-1

6.8 Histology Procedures

Northwest Zoopath will receive the preserved (NBF) whole, fish for examination. Board-certified veterinary pathologist Dr. Michael Garner will conduct external examinations of all whole preserved fish. Sample number (from NBF-filled container) will be recorded by the pathologist for each fish and tissue examined. Each fish will be carefully examined for gross external lesions or abnormalities/tumors with particular attention to any abnormalities of the gills and lateral line. Results of each fish examination will be tabulated by sample number using Northwest ZooPath forms and/or database methods.

If any external lesions (tumors/abnormalities of the skin, lateral line, gills) are identified by the pathologist these will be excised, embedded, stained and examined microscopically. Results of all examinations will be noted in a tabular summary noting (i) type of lesion/abnormality and pathologists observations; (ii) frequency per fish (number) and (iii) severity of each lesion (based upon assigned lesion score).

<u>Lesion Severity</u>	<u>Lesion Score Assigned</u>
No lesion	0
Minimal lesion	1
Mild lesion	2
Moderate lesion	3
Marked lesion	4
Severe lesion	5

The frequency of lesions will also be identified by the pathologist. Representative microphotographs will be taken to illustrate what the pathologist identifies as meeting the lesion types (external, excised tissue) identified above.

An opinion on whether the lesion may be attributed to asbestos fiber exposure will be rendered by the pathologist.

To facilitate later statistical analysis, each fish lesion (if there are any) will be “scored” and the resulting score multiplied by a “pathos factor” of either 1 or 2, recorded by the pathologist, to address if each observed lesion is believed, in the pathologist’s opinion, to potentially be attributable to asbestos fiber exposure (factor of 2), or attributable to other (non-asbestos or natural) causes (factor of 1).

Lesions that occur naturally in reference (control) animals (if any) will be useful in evaluating and considering attribution for animals collected from the asbestos impacted creeks. If an observed lesion is considered to be of natural origin (i.e., parasite, disease) the lesion score will be multiplied by a “pathos” factor of 1. A Pathos factor of 1 indicates no attribution of the lesion is considered likely in the pathologist’s opinion to be associated with exposure to asbestos fibers. A Pathos factor of 2 indicates a

potential or suspected likelihood that the observed lesion or abnormality may be related to asbestos fiber exposure (and all reasoning presented).

The pathologist's report (with microphotographs) and conclusions will clearly indicate whether observed effects, if any, are, in the pathologist's judgment, potentially related to asbestos fibers or other (natural, unknown) causes. The complete draft pathology report will be provided electronically to Golder. Golder will include the complete pathology report as an appendix to the larger study data report, which will be submitted to EPA upon completion.

6.9 Shipping Coolers

Packing and shipping of coolers is done consistent with SOP No. 8 – *Sample Handling and Shipping* (see OU3 eRoom for SOP). Following Quality Checks of cooler contents (which should be done daily following fish processing), COC forms will be filled in to completely inventory/identify cooler contents. The COC will be signed after it is quality checked, bottom copy retained (if duplicate) and the original placed in a large, zip-top baggie taped to the inside of the cooler lid. The cooler will be taped shut on both ends using strapping tape and add a custody seal.

The cooler will be taken to UPS (Libby, MT) and shipped for second or third day delivery to Northwest ZooPath:

Northwest ZooPath
654 W. Main
Monroe, WA
98272
Attn: Dr. Michael Garner

6.10 Data Backup and Submissions to EPA

Once the fish study is completed, pages from the field logbook, Fish Information Forms, and lab notebook pages should be scanned and uploaded to the OU3 eRoom. This is accomplished by going to the Grace office in Libby with a laptop, scanning the required pages using the office copy/scanner and then uploading the data to the following web address: <https://team.cdm.com/eRoom/mt/LibbyOU3>. Individual eRoom access codes are assigned to Golder staff working on the project and these access codes will be used by individuals uploading data. Digital photos taken to document fish collection and processing should be downloaded from the camera to a laptop as a secondary backup and uploaded to the OU3 eRoom along with the other field documentation.

Questions or problems with data uploading to the OU3 eRoom should be directed to Lynn Woodbury (CDM Smith): woodburyl@cdmsmith.com (303) 383-2382.

7.0 References Cited

Agency for Toxic Substances and Disease Registry (ATSDR). 2006. Asbestos Exposure and Your Health. http://www.atsdr.cdc.gov/asbestos/asbestos_exposure_health/asbestos.html.

Bryant, M.D. 2000. Estimating Fish Populations by Removal Methods with Minnow Traps in Southeast Alaska Streams. *North American Journal of Fisheries Management*, 20:923-930.

Parametrix. 2009a. Final Data Report: Remedial Investigation, Operable Unit 3 of the Libby Asbestos Superfund Site, Phase II, Part C: Autumn 2008 Aquatic Data Collection Program. Prepared by Parametrix for Remedium Group, Inc. March 2009.

Parametrix. 2009b. Toxicity of Asbestos in Waters from the Libby Superfund Site Operable Unit 3 (OU3) to Rainbow Trout (*Oncorhynchus mykiss*). Prepared by Parametrix for Remedium Group, Inc. March 2009.

Parametrix. 2010. Final Data Report: Remedial Investigation, Operable Unit 3 of the Libby Asbestos Superfund Site, Phase III: Autumn 2009 Aquatic Data Collection Program. Prepared by Parametrix for Remedium Group, Inc. March 2010.

Figure 1. Minnow trap.



ATTACHMENT A
LIBBY OU3: PHASE V SAP, PART B, REV 0
FISH COLLECTION PERMIT

**MONTANA FISH, WILDLIFE & PARKS
SCIENTIFIC COLLECTORS PERMIT
FISHERIES**

Permit Number:	02-2012	Date Issued:	4/2/2012
Fee Received:	Yes	Date Expires:	12/31/2012
Permit Issued to:	Robinson, Sue 18300 NE Union Hill Road Redmond WA 98052		
Associated With:	Golder Associates Inc., Environmental Consulting Firm		
Associates Name:	Jeremy Clark		

Permission is given to take, kill, capture, or possess, in accordance with the provisions of Section 87-2-806, MCA, the following:

Authorized to use electrofishing, block nets and modified minnow traps to collect up to 10 trout (representing any of the various species present) from each of the two size categories (<65 mm, >65 mm - < 100 mm) from both Upper and Lower Rainy Creeks on the Libby Asbestos Mine Site, and at each of the two off-site reference locations: Bob Tail Tributary and Noisy Creek. Fish shall be euthanized and may be retained for developmental examinations and possible histopathological evaluation. Therefore, a maximum of 40 fish (mix of species) may be collected from the Mine Site (OU3), and a maximum of 40 fish collected from the two reference locations (maximum sample sizes are inclusive of two size categories). Sampling may begin on August 15, 2012 and continue until all fish have been collected. All fish larger than 100 mm must be returned live to the stream near the point of capture. In addition, up to 10 adult trout (of any size) may also be collected from the OU3 on-site Tailings Pond and submitted for analysis of asbestos to support the human health risk assessment being conducted by USEPA Region 8.

Dipnets may also be used to collect amphibians (western toads, Columbia spotted frogs, northern tree frogs) at multiple stages of their development ranging from eggs to metamorphosed juveniles. These organisms may be collected at four ponds (Fleetwood, Carney, Tailings, and Mill Ponds) located on the Libby Mine Site (OU3), and from 3 reference ponds including Bob Tail ponds on private property, and two other ponds yet to be identified. Animals must be euthanized and may be submitted for examinations and histology to assess potential adverse effects. The ponds may be monitored at least weekly in April and May for egg masses or other signs of breeding. Once egg masses are found at each site (OU3, reference), the collection locations may be surveyed and up to 4 egg masses per species per area may be collected. The ponds may be revisited in late spring and early summer (May- early July) and up to 40 tadpoles per species per site in Gosner stages 21-25 and 37-40 may be collected and external development examined. Up to 20 metamorphosed juveniles may be collected per species per site later in July following full metamorphosis.

Final report must be submitted directly to Mike Hensler at the Libby Montana Fish, Wildlife & Parks (FWP) office at the same time the report is submitted to the Helena office.

The permit holder shall follow the department electrofishing guidelines attached to this permit.

When moving to or from all areas, collectors must take measures to disinfect and clean all gear

and equipment between waterbodies (see attached protocol). No live organisms can be transported away from the location of capture without FWP authorization.

REPORT: Upon expiration of the permit, please submit an electronic report containing the permit number, dates, location, and number and kind of specimens collected and released or killed. This form can be downloaded from the FWP website at <http://fwp.mt.gov/fishing> and emailed to beginnings@mt.gov.

By: 
Bruce Rich, Fisheries Bureau Chief
Montana Fish, Wildlife and Parks

Regional Fish Manager
c: Regional Fish Biologist

ATTACHMENT B

Page ___ of ___

Fish Information Form Libby OU3, Fish Population Study

Station _____

Date _____

Sampling Gear and Effort (circle all methods that apply, circle capitalized words in parenthesis that apply)

Boat shocking (DAY / NIGHT) - | Seconds _____ Amps _____ Range (H or L) % of range _____

Barge Shocking _____ | PPS and Mode _____ Str. Length (Meters) _____ Str. Ave. Width (Meters) _____

Backpack | Seconds _____ Str. Length _____ Str. Ave. Width _____ Amps _____ Volts _____ Freq _____ Width _____

Seine | Seine Length (in Meters) _____ Bag (Y or N) Number of Hauls _____

Gillnet | (DAY / NIGHT / OVERNIGHT) Ave. Net Length (in Meters) _____ # Nets Set _____ Ave. Set Time (Hrs.) _____ Mesh Description _____

Fish No.	Species	Total Length (mm)*	Weight (g)	Sex	External Abnormalities, Yes or No
1	_____	_____	_____	_____	_____
2	_____	_____	_____	_____	_____
3	_____	_____	_____	_____	_____
4	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____
6	_____	_____	_____	_____	_____
7	_____	_____	_____	_____	_____
8	_____	_____	_____	_____	_____
9	_____	_____	_____	_____	_____
10	_____	_____	_____	_____	_____
11	_____	_____	_____	_____	_____
12	_____	_____	_____	_____	_____

Sex Notes: M = Male, F = Female, U = Unknown.

Field Data Entered by: _____

Database Entry: _____

Database QC: _____

APPENDIX A.5
Protocol for the Fish Tissue Collection Study
(Revision 0 – July 20, 2012)

APPENDIX A.5

Protocol for the Fish Tissue Collection Study

(Revision 0 – July 20, 2012)

1.0 Introduction

This protocol describes the standard operating procedures (SOPs) and sampling methods to be used when collecting fish at Operable Unit 3 (OU3) for the purposes of tissue burden analysis.

2.0 Health and Safety

All field personnel engaged in the conduct of these trout studies must follow health and safety protocols described in their firm's health and safety plan (and that reflect the nature of the specific work being accomplished). Asbestos fibers are easily inhaled when disturbed and when embedded in the lung tissue can cause health problems. Significant exposure to asbestos increases the risk of lung cancer, mesothelioma, asbestosis (non-cancerous lung disease), and other respiratory diseases (ATSDR 2006). Accordingly, in addition to other health and safety procedures established in a firm's health and safety plan, all field personnel working on OU3 will wear protective garments and respiratory protection appropriate to HAZWOPER protection Level C. Respiratory protection will be via Positive Air Pressure Respirators (PAPRs).

3.0 Background

Historic mining and milling operations at OU3 have resulted in the release of LA to the environment, including releases into several streams and ponds within OU3. Although the exposure pathway of primary concern for humans is inhalation of LA, some studies in animals suggest that ingestion of asbestos fibers can result in the growth of benign intestinal polyps (National Toxicology Program [NTP] 1985). There are several potential scenarios where humans could be exposed to LA via ingestion. One such scenario is the ingestion of edible fish tissue (fillets) from fish that were caught from waters that are contaminated with LA. However, at present, there are no data available on the potential concentrations of LA in fish tissue in OU3 or other waters that may contain LA. Thus, data are needed that can be used to determine the potential for uptake of asbestos fiber into fish tissues.

This protocol is focused on the specific methods and procedural details regarding the field collection of fish from OU3 for tissue analysis. Sample processing (i.e., filleting) and tissue analysis will be not be performed by Remedium contractors.

4.0 Definitions

LA: Libby amphibole

GPS: Global Positioning System

5.0 Responsibilities

This section presents a brief definition of field roles and the responsibilities generally associated with them. This list is not intended to be comprehensive and often additional personnel may be involved. Project team member information shall be included in project-specific plans (e.g., Sampling and Analysis Plan, etc.), and field personnel shall always consult the appropriate documents to determine project-specific roles and responsibilities. In addition, one person may serve in more than one role on any given project.

Project Manager: Selects site-specific sampling methods, sample locations, and constituents to be analyzed with input from other key project staff.

Quality Control Manager: Overall management and responsibility for quality assurance and quality control (QA/QC). Selects QA/QC procedures for the sampling and analytical methods, performs project audits and ensures that data quality objectives are fulfilled.

Field QA Officer: Performs audits of field activities being performed against procedures established in project SOPs for field work activities.

Field Team Leader (FTL): Implements the sampling program, supervises other sampling personnel. Also ensures compliance with SOPs and QA/QC requirements. Prepares daily logs of field activities.

Field Technicians (or other designated personnel): Assist the FTL in the implementation of field tasks. Perform the actual study activities, with the FTL, including study implementation (study set up, monitoring, sample collection, packaging, and documentation (e.g., sample label and log sheet, chain-of-custody record, etc)).

6.0 Fish Field Collection Study Procedures

The remainder of this section contains a detailed summary of the equipment and procedures for conducting the field collection of fish for tissue burden analysis.

6.1 Equipment

The following is a list of equipment that will be needed to conduct the resident trout field collection study.

GENERAL

- Global Position System (GPS) unit
- Clipboard
- Field logbook
- Three-ring binder to store necessary forms used to record and track samples collected at the site. Binders will contain the Fish Information Forms.
- Rite In The Rain® paper
- Permanent marking pen – used to record information in field notebooks and data sheets.
- Digital camera
- Sharpie® brand markers (black)
- Ink pens
- Sample labels (adhesive)
- OU3 Chain of Custody (COC) Forms (carbon copy triplicate)
- Scientific Collection Permit (see **Attachment A**)
- Coolers
- Blue Ice
- Strapping tape
- Custody seals

FISHING

- Backpack electroshocker unit
- Spare Battery
- Waders
- Wader belts
- Set nets (e.g., gill nets)
- Canoe
- Fiberglass handled dip nets

- Fish measuring board – Fish measuring board, in millimeters.
- Pesola hanging scale (for fish weight)
- Trash Bag - used to dispose of gloves and any other non-hazardous waste generated during sampling.
- Foil (to wrap dead fish)
- Blue ice (to store with fish in cooler)

6.2 Collection Location

Of the waters that support fish and are likely to contain LA, the OU3 on-site tailings impoundment best meets these two requirements: concentrations of LA in water are relatively high, and large fish are present. Other locations (e.g., LRC, Carney Creek, Fleetwood Creek) have elevated levels of LA in water, but the sizes of the fish present are relatively small. Fish caught from the Kootenai River below the confluence with Rainy Creek may be large, but water concentrations of LA in Kootenai River are much lower than in on-site OU3 waters. Therefore, even though access to the tailings impoundment and other OU3 waters is currently restricted to include only authorized personnel, fish will be collected from the tailings impoundment to ensure that fish tissues are at the high-end of the potential range of tissue concentrations.

6.3 Sample Collection Goals

Any fish collected will be under the auspices of a scientific collection permit, obtained from Montana Fish Wildlife and Parks (MFWP) (**Attachment A**). The goal of the collection program is to collect 5-10 individual fish (cutthroat, rainbow, or “cut-bow” hybrid trout) of a minimum length of 8 inches.

6.4 Field Sampling Methods

Fish will be collected by netting (e.g., gill nets) and by electro-shocking (with block nets). If necessary a canoe will be used to set nets in deeper parts of the pond. The electro-shocking unit will be a backpack type with positive current. The electro-shocking operator is trained for this activity in the State of Montana.

Any fish caught that are not within the required size needs will be released. Fish will be kept in a bucket (no water) until expired. The fresh fish will be weighed with a hanging scale, wrapped in foil, placed in a zip-top bag labeled with the appropriate sample number (see Section 6.6), and placed in a cooler with Blue Ice. Fish collected will be recorded in the field logbook. The field logbook should specify the collection date, collection method, species, length, and weight for each fish.

The cooler should be hand-delivered to the CDM Smith field office in Libby for subsequent shipment to the TEM analytical laboratory for sample preparation (i.e., filleting) and analysis.

6.6 Assigning Fish Sample Numbers

Sample numbers are manually recorded using Sharpie marker on the outside of the zip-top bag and covered with clear packaging tape. Sampling numbers will be individually assigned to each collected fish as TP-FISH-01, TP-FISH-02, etc.

6.7 Data Backup and Submissions to EPA

Once the fish study is completed, pages from the field logbook pages should be scanned and uploaded to the OU3 eRoom. This is accomplished by going to the Grace office in Libby with a laptop, scanning the required pages using the office copy/scanner and then uploading the data to the following web address: <https://team.cdm.com/eRoom/mt/LibbyOU3>. Individual eRoom access codes are assigned to Golder staff

working on the project and these access codes will be used by individuals uploading data. Digital photos taken to document fish collection should be downloaded from the camera to a laptop as a secondary backup and uploaded to the OU3 eRoom along with the other field documentation.

Questions or problems with data uploading to the OU3 eRoom should be directed to Lynn Woodbury (CDM Smith): woodburyl@cdmsmith.com (303) 383-2382.

7.0 References Cited

Agency for Toxic Substances and Disease Registry (ATSDR). 2006. Asbestos Exposure and Your Health. http://www.atsdr.cdc.gov/asbestos/asbestos_exposure_health/asbestos.html.

NTP (National Toxicology Program). 1985. Technical report series no. 295. Toxicology and carcinogenesis studies of chrysotile asbestos (CAS No. 12001-29-5) in F344/N rats (feed studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 86-2551.

ATTACHMENT A
LIBBY OU3: PHASE V-PART B SAP/QAPP (REVISION 2)
FISH COLLECTION PERMIT

**MONTANA FISH, WILDLIFE & PARKS
SCIENTIFIC COLLECTORS PERMIT
FISHERIES**

Permit Number:	02-2012	Date Issued:	4/2/2012
Fee Received:	Yes	Date Expires:	12/31/2012
Permit Issued to:	Robinson, Sue 18300 NE Union Hill Road Redmond WA 98052		
Associated With:	Golder Associates Inc., Environmental Consulting Firm		
Associates Name:	Jeremy Clark		

Permission is given to take, kill, capture, or possess, in accordance with the provisions of Section 87-2-806, MCA, the following:

Authorized to use electrofishing, block nets and modified minnow traps to collect up to 10 trout (representing any of the various species present) from each of the two size categories (<65 mm, >65 mm - < 100 mm) from both Upper and Lower Rainy Creeks on the Libby Asbestos Mine Site, and at each of the two off-site reference locations: Bob Tail Tributary and Noisy Creek. Fish shall be euthanized and may be retained for developmental examinations and possible histopathological evaluation. Therefore, a maximum of 40 fish (mix of species) may be collected from the Mine Site (OU3), and a maximum of 40 fish collected from the two reference locations (maximum sample sizes are inclusive of two size categories). Sampling may begin on August 15, 2012 and continue until all fish have been collected. All fish larger than 100 mm must be returned live to the stream near the point of capture. In addition, up to 10 adult trout (of any size) may also be collected from the OU3 on-site Tailings Pond and submitted for analysis of asbestos to support the human health risk assessment being conducted by USEPA Region 8.

Dipnets may also be used to collect amphibians (western toads, Columbia spotted frogs, northern tree frogs) at multiple stages of their development ranging from eggs to metamorphosed juveniles. These organisms may be collected at four ponds (Fleetwood, Carney, Tailings, and Mill Ponds) located on the Libby Mine Site (OU3), and from 3 reference ponds including Bob Tail ponds on private property, and two other ponds yet to be identified. Animals must be euthanized and may be submitted for examinations and histology to assess potential adverse effects. The ponds may be monitored at least weekly in April and May for egg masses or other signs of breeding. Once egg masses are found at each site (OU3, reference), the collection locations may be surveyed and up to 4 egg masses per species per area may be collected. The ponds may be revisited in late spring and early summer (May- early July) and up to 40 tadpoles per species per site in Gosner stages 21-25 and 37-40 may be collected and external development examined. Up to 20 metamorphosed juveniles may be collected per species per site later in July following full metamorphosis.

Final report must be submitted directly to Mike Hensler at the Libby Montana Fish, Wildlife & Parks (FWP) office at the same time the report is submitted to the Helena office.

The permit holder shall follow the department electrofishing guidelines attached to this permit.

When moving to or from all areas, collectors must take measures to disinfect and clean all gear

and equipment between waterbodies (see attached protocol). No live organisms can be transported away from the location of capture without FWP authorization.

REPORT: Upon expiration of the permit, please submit an electronic report containing the permit number, dates, location, and number and kind of specimens collected and released or killed. This form can be downloaded from the FWP website at <http://fwp.mt.gov/fishing> and emailed to begiddings@mt.gov.

By: 
Bruce Rich, Fisheries Bureau Chief
Montana Fish, Wildlife and Parks

Regional Fish Manager
c: Regional Fish Biologist

Appendix B

Standard Operating Procedures (SOPs)

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APPENDIX B

STANDARD OPERATING PROCEDURES**

SOP ID	SOP Description
No. 3	Surface Water Sampling
No. 5	Sediment Sampling
No. 7	Equipment Decontamination
No. 8	Sample Handling and Shipping
No. 9	Field Documentation
No. 11	GPS Data Collection
No. 12	Investigation Derived Waste (IDW) Management
No. 20	Surface Water Temperature Data Logger

*** The most recent versions of all SOPs are provided electronically in the OU3 eRoom (<https://team.cdm.com/eRoom/mt/LibbyOU3>).*

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Appendix C

FSDS Forms

The most recent versions of FSDS forms are provided electronically in the OU3 eRoom (<https://team.cdm.com/eRoom/mt/LibbyOU3>).

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Appendix D

COC Form

The most recent versions of COC forms are provided electronically in the OU3 eRoom (<https://team.cdm.com/eRoom/mt/LibbyOU3>).

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Appendix E

Record of Modification Forms (ROMs)

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FIELD MODIFICATION APPROVAL FORM
LFM-OU3-xx
Libby OU3 Phase V Part B Sampling & Analysis Plan

Requested by: _____

Date: _____

Description of Deviation:

☐ EPA Region 8 has reviewed this field modification approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

Christina Progross, EPA RPM

Date

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Request for Modification
to
Laboratory Activities
LB-0000XX

Instructions to Requester: E-mail form to contacts at bottom of form for review and approval.

All Labs Applicable Forms – copies to: EPA LC, QATS contractor, All Project Labs

Individual Labs Applicable Forms – copies to: EPA LC, QATS contractor, Initiating Lab

Method (**circle all applicable**):
EPA/600/R-93/116 TEM-AHERA TEM-ISO 10312 PCM-NIOSH 7400
ASTM 5755 TEM 100.2 SRC-LIBBY-03
SRC-LIBBY-01 NIOSH 9002 Other: _____

Requester: _____ Title: _____
Company: _____ Date: _____

Original Requester: _____ Original Request Date: _____
[only applicable if modification is a revision of an earlier modification]

Description of Modification:

Reason for Modification:

Potential Implications of this Modification:

Laboratory Applicability (**circle one**): **All** **Individual(s)** _____

This laboratory modification is (**circle one**): **NEW** **APPENDS to** _____ **SUPERCEDES** _____

Duration of Modification (circle one):
Temporary Date(s): _____
Analytical Batch ID: _____
Temporary Modification Forms – Attach legible copies of approved form with all associated raw data packages

Permanent (Complete Proposed Modification Section) Effective Date: _____
Permanent Modification Forms – Maintain legible copies of approved form in a binder that can be accessed by analysts.

Proposed Modification to Method (attach additional sheets if necessary; state section and page numbers of method when applicable):

REFERENCES

Data Quality Indicator (**circle one**) – Please reference definitions below for direction on selecting data quality indicators:

Not Applicable

Reject

Low Bias

Estimate

High Bias

No Bias

DATA QUALITY INDICATOR DEFINITIONS:

Reject - Samples associated with this modification form are not useable. The conditions outlined in the modification form adversely affect the associated sample to such a degree that the data are not reliable.

Low Bias - Samples associated with this modification form are useable, but results are likely to be biased low. The conditions outlined in the modification form suggest that associated sample data are reliable, but estimated low.

Estimate - Samples associated with this modification form are useable, but results should be considered approximations. The conditions outlined in the modification form suggest that associated sample data are reliable, but estimates.

High Bias - Samples associated with this modification form are useable, but results are likely to be biased high. The conditions outlined in the modification form suggest that associated sample data are reliable, but estimated high.

No Bias - Samples associated with this modification form are useable as reported. The conditions outlined in the modification form suggest that associated sample data are reliable as reported.

Technical Review: _____ Date: _____
(Laboratory Manager or designate)

Project Review and Approval: _____ Date: _____
(USEPA: Project Manager or designate)

Approved By: _____ Date: _____
(USEPA: Technical Assistance Unit Chief or designate)



Request for Modification To Soil Sample Preparation Activities

**Instructions to Requester: E-mail form to contacts at bottom of form for review and approval.
File approved copy at the Sample Preparation Facility (SPF).**

Requester: _____ Title: _____

Company: _____ Date: _____

Effective Date: _____

Description of Modification:

Reason for Modification:

Potential Implications of this Modification:

Duration of Modification (circle one):

Temporary Date(s): _____
Preparation Batch ID: _____

- Temporary Modification Forms – Attach legible copies of approved form with all associated chain-of-custody forms. Also, maintain legible copies of approved form in a binder that can be accessed by SPF personnel.

Permanent (complete Proposed Modification to Method)

- Permanent Modification Forms – Maintain legible copies of approved form in a binder that can be accessed by CSF personnel.

Proposed Modification to Method (attach additional sheets if necessary; state section and page numbers of Method when applicable):

Technical Review: _____ Date: _____
(SPF Manager or designate)

Approved By: _____ Title: _____ Date: _____
(USEPA: Project Chemist or designate)

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Appendix F

Analytical Requirements Sheet

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SAP ANALYTICAL SUMMARY # OU3PHVB-0412
SUMMARY OF PREPARATION AND ANALYTICAL REQUIREMENTS FOR ASBESTOS

SAP Title: Phase V Sampling and Analysis Plan for Operable Unit 3, Libby Asbestos Superfund Site - Part B: 2012 Ecological Studies

SAP Date/Revision: May 22, 2012 (Rev. 1)

EPA Technical Advisor: Christina Progross (303-312-6009, progross.christina@epa.gov)
 (contact to advise on DQOs of SAP related to preparation/analytical requirements)

Sampling Program Overview: The purpose of Part B of the Phase V SAP/QAPP for OU3 is to guide the collection of several ecological studies to evaluated potential impacts of LA to fish and amphibians at OU3. This summary includes the asbestos analyses that are the responsibility of OU3 (i.e., Remedium).

Estimated number and timing of field samples (does not include field QC):

Amphibian Toxicity Test --

>> Sediment (late April): 30 samples

>> Sediment (late June): 4 samples

Amphibian Field Study --

>> Sediment = 14 samples (late April), 8 samples (mid-May), 8 samples (late August)

>> Surface water (May - August, 16 weeks) = 4-8 samples per week

Caged Fish Studies --

>> Eyed Egg Study: Water (May - June, 8 weeks) = 8-14 samples per week

>> Fry Study: Surface water (May - June, 4 weeks) = 8 samples per week

Index ID Prefix: P5-2xxxx

PLM Preparation and Analytical Requirements for Sediment Samples:

Medium Code	Medium	Preparation Method ^[a]	Analysis Method ^[b]	Applicable Laboratory Modifications
B	Sediment	ISSI-LIBBY-01 Rev. 11	PLM-Grav: SRC-LIBBY-01 Rev. 3 PLM-VE: SRC-LIBBY-03 Rev. 3	N/A

[a] Sample preparation to be performed at the Troy sample preparation facility and shipped to the PLM analytical laboratory.

[b] After sample preparation, multiple aliquots will be generated for each sample. The analytical laboratory should do the following for each aliquot:

A (archive) – place sample in archive

C (coarse) – analyze sample by PLM-Grav

FG1 (fine ground aliquot #1) – analyze sample by PLM-VE

FG2-4 (fine ground aliquots #2 to #4) – place samples in archive

TEM Preparation and Analytical Requirements for Water Samples:

Medium Code	Medium	Preparation Details ^[c]				Analysis Details			Applicable Laboratory Modifications (current version of)
		Investigative?	Indirect Prep?		Filter Archive?	Method	Recording Rules	Analytical Sensitivity/ Stopping Rules	
			With Ashing	Without Ashing					
A	Water	Yes	No	No	Yes	Standard TEM; ISO 10312	All asbestos ^[d] ; L: $\geq 0.5 \mu\text{m}$ AR: $\geq 3:1$	Count a minimum of 2 grid openings in 2 grids, then continue counting until one is achieved: i) sensitivity of 50,000 L ⁻¹ is achieved ii) 25 structures are recorded iii) A total filter area of 1.0 mm ² has been examined (approx. 100 grid openings)	LB-000016, LB-000029, LB-000066, LB-000067, LB-000085

[c] Sample and filter preparation should be performed in basic accordance with EPA Method 100.2 (as modified by LB-000020A). Grid preparation should be performed in basic accordance with Section 9.3 of ISO 10312:1995(E).

[d] If observed, chrysotile structures will not be recorded, but the presence of chrysotile structures should be recorded in the analysis comments.

Laboratory Quality Control Sample Frequencies:

PLM [e]: Lab Duplicates – 10% (cross-check 8%; self-check 2%)

Inter-laboratory – 1% [f]

[e] See SRC-LIBBY-03 for selection procedure and QC acceptance criteria.

[f] *Post hoc* selection to be performed by the QATS contractor.

TEM [g]: Lab Blank – 4%

Recount Same – 1%

Verified Analysis – 1%

Repreparation – 4%

Recount Different – 2.5%

Inter-laboratory - 2% [h]

[g] See LB-000029 for selection procedure and QC acceptance criteria.

[h] *Post hoc* selection to be performed by the QATS contractor.

Requirements Revision:

Revision #:	Effective Date:	Revision Description
0	4/20/12	--
1	5/2/12	Add footnote to PLM table to explicitly state what should be done by the analytical laboratory with each aliquot (A, C, FG) generated by the Troy SPF.
2	5/22/12	Change associated SAP/QAPP to Revision 1.

Asbestos Analytical Laboratory Review Sign-off:

All laboratories signed the original version of this analytical summary sheet (Rev0); this revision did not require another signature process.

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Appendix G
Asbestos Laboratory Acceptance Criteria for
Libby Asbestos Superfund Site

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APPENDIX G
ASBESTOS LABORATORY ACCEPTANCE CRITERIA
FOR LIBBY ASBESTOS SUPERFUND SITE

MINIMUM LABORATORY ACCEPTANCE CRITERIA

1. Must be certified by the National Institute of Standards and Technology (NIST) National Voluntary Laboratory Accreditation Program (NVLAP) for the analysis of asbestos by PLM¹ and/or TEM².
2. Must have a laboratory-specific Quality Management Plan and all relevant SOPs in place for asbestos environmental sample processing and analysis.
3. Must have multiple experienced analysts on staff capable of running PLM visual area estimation methods [NIOSH 9002, EPA 600] and/or TEM methods [ISO 10312, ISO 13794, AHERA, ASTM 5755, EPA Method 100.2] (a minimum of 2 analysts within each laboratory are needed to assess within-laboratory reproducibility). Must have documentation in place demonstrating all analysts work experience and training related to analyses performed.

4. Must be familiar with standard TEM and PLM preparation methods. TEM laboratories must have ability to perform indirect preparation and ashing (for the analysis of air, dust, other media) and/or ozonation/UV/sonication treatment (for the analysis water). PLM laboratories must have the ability to dry samples (for PLM-NIOSH 9002 analysis). If the PLM laboratory wishes to perform soil sample preparation in support of the Libby-specific PLM methods (i.e., PLM-VE and PLM-Grav), the laboratory must have the ability to sieve and grind soil samples in accordance with the Libby-specific preparation method.

Note: Not all laboratory facilities need to have all preparation capabilities; media analysis could be segregated based on facility capability (i.e. one laboratory does water, another does soil, etc.).

5. TEM laboratories must have Energy Dispersive Spectroscopy (EDS) and Selected Area Electron Diffraction (SAED) capability incorporated into their microscope(s).
6. Must participate in monthly EPA laboratory calls for the Libby project.
7. Must participate in inter-laboratory analyses with other Libby project laboratories.
8. Must participate in annual EPA (QATS) audits and in other laboratory and/or data audits if data quality issues arise, as deemed appropriate by EPA.
9. Must be capable of using Libby-specific bench sheets to record observations and utilizing Libby-specific electronic data deliverables (EDDs) to report analytical results.
10. Must have the capacity to meet the required delivery schedules and turn-around times.
11. Must designate laboratory primary and secondary points of contact for discussion of EPA/laboratory issues.

EPA APPROVAL PROCESS

1. Once potential laboratories are identified that meet the minimum acceptance criteria, they must show proficiency in analysis of NIST/NVLAP performance evaluation samples and inter-laboratory samples

¹ <http://www.nist.gov/nvlap/upload/NIST-HB-150-3-2006-1.pdf>

² <http://www.nist.gov/nvlap/upload/NIST-HB-150-13-2006-1.pdf>

(standard PLM visual area estimation and TEM only, no Libby-specific method modifications and requirements).

2. If proficiency is documented, an EPA (QATS) audit will be performed.
3. If any deficiencies found during the audit are sufficiently resolved to EPA's satisfaction, then project-specific mentoring will be conducted to ensure laboratories are proficient in the Libby-specific methods, modifications, and requirements.
4. Once a laboratory has passed all of these steps, EPA will approve the use of the laboratory and documentation to this effect will be sent to the laboratory. Samples can then be sent to the laboratory for analysis.